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(54) **ANTIBODY AGAINST N-TERMINAL PEPTIDE OR C-TERMINAL PEPTIDE OF GPC3
SOLUBILIZED IN BLOOD**

(57) Disclosed is an antibody against a secreted form of GPC3 capable of detecting a secreted form of glypican 3 (GPC3) in a test sample. It is possible to determine whether a subject suffers from cancer, in particular hepatoma. Also disclosed is an antibody against

GPC as well as a cell disrupting agent and an anti-cancer agent comprising the same, which can disrupt cells, in particular cancer cells.

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Description

Technical Field

5 [0001] The present invention relates to an antibody against an N-terminal peptide or C-terminal peptide of GPC3. More specifically, the invention relates to an antibody against a GPC3 N-terminal peptide of about 40 kDa as found in the soluble form of the GPC3 core protein. Additionally, the invention also relates to an antibody against a GPC3 C-terminal peptide of about 30 kDa as found in the soluble form of the GPC3 core protein.

10 Background Art

[0002] The presence of the glypican family is reported as a new family of heparan sulfate proteoglycan existing on cell surface. Up to now, it is reported that five types of glypican (glypican 1, glypican 2, glypican 3, glypican 4 and glypican 5) exist. The members of the family have a core protein of a uniform size (about 60 kDa) and have unique
15 cysteine residues well conserved in common, and are bound to cell membrane via glycosylphosphatidylinositol (GPI) anchor.

[0003] Glypican 3 (GPC3) is known to be deeply involved in cell division during development and the control of the pattern thereof. Additionally, it is known that the GPC3 gene is highly expressed in hepatoma cell and that the GPC3 gene is possibly used as a marker of hepatocellular carcinoma.

20 [0004] The present inventors previously found that an anti-GPC3 antibody had an ADCC activity and a CDC activity and was useful as the therapeutic treatment of hepatoma and filed a patent application (Japanese Patent Application 2001-189443).

[0005] However, GPC3 is a membrane-bound protein and it has not been reported that a GPC3 protein of secreted form existed. Thus, no examination has been made about the use of the GPC3 protein itself as a tumor marker in blood.
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Disclosure of the Invention

[0006] The present inventors found a fact that glypican 3 (GPC3) is cleaved at an amino acid residue 358 thereof or at an amino acid residue 374 thereof or a region in the vicinity of the residues. On an assumption that the soluble form
30 of GPC3 would be secreted in the blood of hepatoma patients, the inventors established a GPC3 sandwich ELISA system to show the existence of the secreted form of GPC3 in the culture supernatant of human hepatoma cell HepG2 highly expressing GPC3. Further, the inventors successfully assayed the secreted form of GPC3 not only in the plasma of a mouse transplanted with HepG2 but also in the serum of a human hepatoma patient. Because the expression of the GPC3 gene is observed in hepatoma at an earlier stage compared with the time involving the occurrence of AFP
35 as a hepatoma marker, the inventors considered that the detection of GPC3 would be useful for cancer diagnosis. Additionally because it appears to be hard to detect the secreted form of GPC3 with an anti-GPC3 antibody recognizing a C-terminal peptide fragment, the secreted form of GPC3 was assumed to be dominantly present as an N-terminal peptide fragment. Thus, the inventors considered that an anti-GPC3 antibody recognizing the N terminus was preferably used for detecting the secreted form of GPC3. Accordingly, the inventors made an attempt to develop an antibody
40 recognizing the N-terminal peptide of GPC3, and thus have achieved the invention. Further, the inventors found that an antibody against the C terminus of GPC3 had a high cytotoxic activity and considered that the use of the anti-GPC3 antibody recognizing the C terminus would be preferable for disrupting cancer cell, i.e. for therapeutically treating cancer. Then, the inventors made an attempt of developing an antibody recognizing the C-terminal peptide of GPC3, and thus have achieved the invention.

45 [0007] Since it is observed that GPC3 is expressed in cancer cell lines other than hepatoma cell lines, such as lung cancer, colon cancer, breast cancer, prostate cancer, pancreatic cancer, and lymphoma, GPC3 may possibly be applied to the diagnosis of cancers other than hepatoma.

[0008] Specifically, the invention relates to an antibody against an N-terminal peptide of GPC3.

[0009] Additionally, the invention relates to the antibody, where the N-terminal peptide of GPC3 is a secreted form
50 of a peptide found in blood.

[0010] Further, the invention relates to the antibody, where the N-terminal peptide of GPC3 is a peptide comprising amino acid residues 1-374 of GPC3 or a peptide comprising amino acid residues 1-358 of GPC3.

[0011] Still further, the invention relates to the antibody, which is a monoclonal antibody.

[0012] Additionally, the invention relates to the antibody, which is immobilized to an insoluble support.

55 [0013] Still additionally, the invention relates to the antibody, which is labeled with a labeling material.

[0014] Still more additionally, the invention relates to an antibody against a C-terminal peptide of GPC3.

[0015] Still further, the invention relates to the antibody, where the C-terminal peptide of GPC3 is a peptide comprising amino acid residues 359-580 of GPC3 or a peptide comprising amino acid residues 375-580 of GPC3.

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- [0016] Still further, the invention relates to the antibody, which is a monoclonal antibody.
- [0017] Additionally, the invention relates to the antibody, which is a chimera antibody.
- [0018] Additionally, the invention relates to the antibody, which is a cytotoxic antibody.
- [0019] Still additionally, the invention relates to a cell-disrupting agent comprising the antibody.
- 5 [0020] Additionally, the invention relates to the cell disrupting agent, where the cell is a cancer cell.
- [0021] Further, the invention relates to an anti-cancer agent comprising the antibody.
- [0022] Additionally, the invention relates to a method for inducing cytotoxicity comprising contacting a cell with the antibody.
- [0023] Still more additionally, the invention relates to the method, where the cell is a cancer cell.
- 10 [0024] The invention is now described in detail hereinbelow.
- [0025] The invention provides an antibody against the secreted form of glypican 3 (GPC3), which is capable of detecting the secreted form of GPC3 in a test sample. By detecting the secreted form of GPC3 in vitro in a test sample, it can be diagnosed whether or not the test subject is afflicted with cancer, particularly hepatoma.
- [0026] Detection includes quantitative or non-quantitative detection, and includes for example a simple assay for the existence of GPC3 protein, an assay for the existence of GPC3 protein at a given amount or more, and a comparative assay for the amount of GPC3 protein with the amount in other samples (for example, control sample) as a non-quantitative assay; and an assay for the concentration of the GPC3 protein and an assay for the amount of the GPC3 protein as a quantitative assay.
- 15 [0027] The test sample includes, but is not limited to, any samples possibly containing the GPC3 protein. A sample collected from biological bodies of mammals is preferable. Further, samples collected from humans are more preferable. Specific examples of such test sample include blood, interstitial fluid, plasma, extravascular fluid, cerebrospinal fluid, synovial fluid, pleural fluid, serum, lymphoid fluid, saliva, and urine. Preferably, the test sample is blood, serum or plasma. Additionally, samples obtained from test samples, such as a culture medium of cells collected from biological bodies are also included in the test sample in accordance with the invention.
- 20 [0028] The cancer to be diagnosed using the antibody against the N-terminal peptide of GPC3 in accordance with the invention includes, but is not limited to, hepatoma, pancreatic cancer, lung cancer, colon cancer, breast cancer, prostate cancer, leukemia, and lymphoma. Preferably, the cancer is hepatoma.
- [0029] Because the antibody against the C-terminal peptide of GPC3 in accordance with the invention has a high cytotoxic activity, the antibody can be used for disrupting cancer cells, i.e. for therapeutically treating cancer. Cancer possibly treated clinically using the antibody includes, but is not limited to, hepatoma, pancreatic cancer, lung cancer, colon cancer, breast cancer, prostate cancer, leukemia, and lymphoma. Preferably, the cancer is hepatoma.
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1. Preparation of the anti-GPC3 antibody against the N-terminal peptide or the anti-GPC3 antibody against the C-terminal peptide

- 35 [0030] The amino acid sequence and nucleotide sequence of GPC3 are described in Lage, H. et al., Gene 188 (1997), 151-156 or GenBank: Z37987.
- [0031] The anti-GPC3 antibody against the N-terminal peptide or the anti-GPC3 antibody against the C-terminal peptide used in the invention should be capable of specifically binding to the N-terminal peptide of the GPC3 protein or the C-terminal peptide of the GPC3 protein, respectively. The origin or type thereof (monoclonal, polyclonal) or the shape thereof is not specifically limited. Specifically, known antibodies such as mouse antibody, rat antibody, human antibody, chimera antibody and humanized antibody can be used.
- 40 [0032] When GPC3 is cleaved at a cleavage site, the GPC3 is cut into a peptide of about 40 kDa and a peptide of about 30 kDa, which are on the N-terminal side and the C-terminal side, respectively. The cleavage site of GPC3 is the amino acid residue 358, the amino acid residue 374 or a region in the vicinity thereof. The main cleavage site is believed to be the amino acid residue 358.
- 45 [0033] The N-terminal peptide of GPC3 is an N-terminal peptide of GPC3 and of about 40 kDa, which is found in the soluble form of the GPC3 core protein. The N-terminal peptide is preferably a peptide of an amino acid sequence comprising from Met 1 to Lys 374, or a peptide of an amino acid sequence comprising from Met 1 to Arg 358. More preferably, the N-terminal peptide is a peptide of an amino acid sequence comprising from Met 1 to Arg 358, because the main cleavage site is predicted to be at the amino acid residue 358. In accordance with the invention, fragments of the N-terminal peptide may also be employed. In this specification, the N-terminal peptide is also referred to as N-terminal fragment or N-terminal peptide fragment.
- 50 [0034] In other words, the antibody against the N-terminal peptide of GPC3 in accordance with the invention is an antibody recognizing an epitope existing on the N-terminal peptide of the GPC3 protein. The site of the epitope recognized is not specifically limited.
- 55 [0035] The C-terminal peptide of GPC3 is a C-terminal peptide of GPC3 and of about 30 kDa found in the soluble form of the GPC3 core protein. Based on the cleavage site mentioned above, the C-terminal peptide is preferably a

peptide of an amino acid sequence of from Ser 359 to His 580 or a peptide of an amino acid sequence of from Val 375 to His 580. More preferably, the C-terminal peptide is a peptide of an amino acid sequence comprising from Ser 359 to His 580, because the main cleavage site is presumed to be at the site of the amino acid residue 358. In accordance with the invention, fragments of such C-terminal peptide may also be employed. In this specification, the C-terminal peptide is also referred to C-terminal fragment or C-terminal peptide fragment.

[0036] In other words, the antibody against the C-terminal peptide of GPC3 in accordance with the invention is an antibody recognizing an epitope existing on the C-terminal peptide of the GPC3 protein, and the site of the epitope recognized is not limited.

[0037] The antibody may be a polyclonal antibody but is preferably a monoclonal antibody.

[0038] The anti-GPC3 N-terminal peptide antibody or the anti-GPC3 C-terminal peptide antibody for use in accordance with the invention can be obtained as a polyclonal antibody or a monoclonal antibody, using known techniques. The anti-GPC3 antibody for use in accordance with the invention is preferably a monoclonal antibody derived from mammals. The monoclonal antibody derived from mammals includes those produced by hybridoma, and those generated in hosts transformed with expression vectors carrying the antibody gene by genetic engineering technology.

[0039] Hybridoma producing a monoclonal antibody is prepared essentially using known techniques as follows. An animal is immunized by a conventional immunization method using GPC3 as a sensitizing antigen to obtain an immune cell, which is then fused to a known parent cell by a conventional cell fusion method. Fused cells are screened for monoclonal antibody-generating cells by a conventional screening method.

[0040] Specifically, a monoclonal antibody is prepared as follows.

[0041] First, GPC3 for use as a sensitizing antigen for obtaining antibody is prepared by expressing the GPC3 (MXR7) gene/amino acid sequence disclosed in Lage, H. et al., Gene 188 (1997), 151-156. Particularly, the gene sequence encoding GPC3 is inserted in a known expression vector to transform an appropriate host cell, then the intended human GPC3 protein is purified from the host cell or a culture supernatant thereof.

[0042] Additionally, naturally occurring GPC3 may also be purified and used.

[0043] Then, the purified GPC3 protein is used as a sensitizing antigen. The whole GPC3 protein may be used as a sensitizing antigen. Because an antibody against the N-terminal peptide of the GPC3 protein and an antibody against the C-terminal peptide thereof are also induced in this case, the antibody against the N-terminal peptide of the GPC3 protein and the antibody against the C-terminal peptide thereof may be separately selected. Alternatively, a partial N-terminal peptide of GPC3 or a partial C-terminal peptide thereof may also be used as a sensitizing antigen. In that case, such partial peptide may be obtained by chemical synthesis on the basis of the amino acid sequence of human GPC3 or by inserting a part of the GPC3 gene into an expression vector or by degrading naturally occurring GPC3 with proteases. The part of GPC3 for use as a partial peptide is the N-terminal GPC3 peptide. A smaller peptide fragment containing the epitope in the part may also be used. Further, a C-terminal peptide of GPC3 may be used as a partial peptide, and a smaller peptide fragment containing the epitope in the part may also be used.

[0044] Mammals for immunization with a sensitizing antigen are preferably selected, with taking account of the compatibility with parent cells for use in cell fusion. The mammals used for immunization preferably include, but are not limited to, rodents such as mouse, rat, hamster or rabbit or monkey.

[0045] For immunization of animals with a sensitizing antigen, known methods may be employed. Generally, for example, a sensitizing antigen is injected intraperitoneally or subcutaneously in mammals. Specifically, a sensitizing antigen is diluted with or suspended in PBS (phosphate-buffered saline) or physiological saline or the like, to an appropriate volume, and mixed with an appropriate volume of conventional adjuvants, such as Freund's complete adjuvant. After emulsification, the emulsified mixture is administered to mammals several times every 4 to 21 days. Additionally, an appropriate carrier may be used during the immunization with a sensitizing antigen. In case that a partial peptide of a very small molecular weight is to be used as a sensitizing antigen, the partial peptide may preferably be bound to carrier proteins, such as albumin and keyhole limpet hemocyanin upon immunization.

[0046] After mammals are immunized as above and the increase in the level of a desired antigen in serum is observed, immune cells are collected from the mammals, which are then subjected to cell fusion. Preferably, the immune cell is splenocyte.

[0047] As another parent cell to be fused to the immune cell, mammalian myeloma cell may be used. As the myeloma cell, known various cell lines are preferably used, including for example P3 (P3x63Ag8. 653) (J. Immunol. (1979) 123, 1548-1550), P3x63Ag8U. 1 (Current Topics in Microbiology and Immunology (1978) 81, 1-7), NS-1 (Kohler G. and Milstein, C. Eur. J. Immunol. (1976) 6, 511-519), MPC-11 (Margulies, D. H. et al., Cell (1976) 8, 405-415), SP2/0 (Shulman, M. et al., Nature (1978) 276, 269-270), F0 (de St. Groth, S. F. et al., J. Immunol. Methods (1980) 35, 1-21), S194 (Trowbridge, I. S. J. Exp. Med. (1978) 148, 313-323), and R210 (Galfre, G. et al., Nature (1979) 277, 131-133).

[0048] The cell fusion of the immune cell to the myeloma cell is essentially done by known methods, for example the method of Kohler & Milstein et al. (Kohler G. and Milstein C., Methods Enzymol. (1981) 73, 3-46).

[0049] More specifically, the cell fusion is carried out in conventional nutritious culture media in the presence of a cell fusion stimulator. Cell fusion stimulator includes, for example, polyethylene glycol (PEG) and Sendai virus (HVJ).

If desired, auxiliary agents such as dimethylsulfoxide can be added and used so as to enhance the fusion efficiency.

[0050] The ratio of an immune cell and a myeloma cell to be used can appropriately be determined. For example, an immune cell at a ratio of 1- to 10-fold a myeloma cell is preferable. Culture medium for use in the cell fusion includes, for example, RPMI1640 and MEM, and other conventional culture media suitable for the growth of myeloma cell lines.

Further, auxiliary serum agents such as fetal calf serum (FCS) may be used in combination.

[0051] The cell fusion can be done by thoroughly mixing predetermined amounts of immune cells and myeloma cells in the culture medium, adding the resulting mixture to a PEG solution (for example, mean molecular weight of about 1,000 to 6,000) preliminarily heated to about 37 °C, generally to a concentration of 30 to 60 w/v %, and subsequently mixing the mixture to allow the intended fusion cell (hybridoma) to be formed. Subsequently, a cell fusion agent and the like unpreferable for the growth of hybridoma are removed by adding appropriate culture medium sequentially and centrifuging the mixture to discard the supernatant, and repeating the procedures described above.

[0052] The hybridoma thus obtained is selected by culturing in a conventional selective culture medium, such as HAT medium (containing hypoxanthine, aminopterin and thymidine). The culturing in the HAT medium is continued for a sufficient period of time (typically several days to several weeks) for killing cells (non-fused cells) other than the intended hybridoma cell. Then, a conventional limited dilution method is carried out for screening and single cloning of a hybridoma producing the intended antibody.

[0053] The screening and the single cloning of the hybridoma may be done by a screening method on the basis of known antigen-antibody reactions. The antigen is bound to carriers such as beads made of polystyrene and the like, or commercially available 96-well microtiter plates, and reacted with a culture supernatant of the hybridoma. After rinsing the carriers, an enzyme-labeled secondary antibody is added to the plate to determine whether an intended antibody reacting with the sensitizing antigen is contained in the culture supernatant. The hybridoma producing the intended antibody can be cloned by limited dilution method. The N-terminal peptide of GPC3 or a fragment thereof or the C-terminal peptide of GPC3 or a fragment thereof may be used as the antigen for screening.

[0054] In addition to obtaining hybridoma by immunizing an animal except humans with an antigen, a human antibody may be prepared by another method. Human lymphocyte is sensitized with GPC3 in vitro and is then fused to myeloma cell with a permanent division potency derived from humans, to obtain a desired human antibody with a binding activity to the N-terminal peptide of GPC3 or the C-terminal peptide of GPC3 (see JP-B-1-59878). Further, a human antibody against the N-terminal peptide of GPC3 or the C-terminal peptide of GPC3 may be obtained by administering GPC3 as an antigen to a transgenic animal bearing all the repertoires of the genes of human antibodies to obtain a cell producing an anti-GPC3 antibody against the N-terminal peptide or a cell producing an anti-GPC3 antibody against the C-terminal peptide, and then immortalizing the cell (see International Publications WO 94/25585, WO 93/12227, WO 92/03918, and WO 94/02602).

[0055] The hybridoma producing the monoclonal antibody thus prepared can be subcultured in a conventional culture medium and can be stored in liquid nitrogen for a long period of time.

[0056] One method for obtaining the monoclonal antibody from the hybridoma involves culturing the hybridoma by a conventional method and obtaining the monoclonal antibody from a culture supernatant thereof. Another method involves administering the hybridoma to an animal compatible to the hybridoma for proliferation and obtaining the monoclonal antibody in the form of ascites. The former method is suitable for obtaining the antibody at high purity, while the latter method is suitable for large-scale production of the antibody.

[0057] In accordance with the invention, a monoclonal antibody includes a recombinant antibody produced by gene recombinant technology. A recombinant antibody can be generated by cloning the gene of the antibody from the hybridoma, integrating the gene into an appropriate vector, introducing the gene into a host, and allowing the recombinant antibody to be produced by the host (see for example Vandamme, A. M. et al., Eur. J. Biochem. (1990) 192, 767-775, 1990). Specifically, mRNA encoding the variable (V) region of the anti-GPC3 N-terminal peptide or the anti-GPC3 C-terminal peptide is isolated from the hybridoma generating the anti-GPC3 N-terminal peptide antibody or the hybridoma generating the anti-GPC3 C-terminal peptide antibody, respectively. mRNA isolation can be done by known methods. For example, total RNA is prepared by guanidine ultra-centrifugation method (Chirgwin, J. M. et al., Biochemistry (1979) 18, 5294-5299) or AGPC method (Chomczynski, P. et al., Anal. Biochem. (1987) 162, 156-159), from which the intended mRNA is prepared using the mRNA purification kit (manufactured by Pharmacia). Alternatively, mRNA can directly be prepared using QuickPrep mRNA purification kit (manufactured by Pharmacia).

[0058] cDNA of the V region of the antibody is synthesized from the resulting mRNA, using reverse transcriptase. cDNA can be synthesized, using AMV Reverse Transcriptase First-strand cDNA Synthesis Kit (manufactured by Seikagaku Corporation). cDNA can also be synthesized and amplified using 5'-AmpliFinder Race Kit (manufactured by Clontech) and 5'-RACE method using PCR (Frohman, M.A. et al., Proc. Natl. Acad. Sci. USA (1988) 85, 8998-9002; Belyavsky, A. et al., Nucleic Acids Res. (1989) 17, 2919-2932).

[0059] The intended DNA fragment is purified from the resulting PCR product and linked to vector DNA. A recombinant vector is prepared from the vector DNA and introduced in *Escherichia coli* and the like to select a colony for preparation of a desired recombinant vector. Subsequently, the nucleotide sequence of the intended DNA can be

confirmed by known methods, for example dideoxynucleotide chain termination method.

[0060] After DNA encoding the V region of the intended anti-GPC3 N-terminal peptide antibody or the intended anti-GPC3 C-terminal peptide antibody is obtained, the DNA is inserted into an expression vector containing DNA encoding the desired constant region (C region) of the antibody.

[0061] So as to produce the anti-GPC3 N-terminal peptide antibody or the anti-GPC3 C-terminal peptide antibody for use in accordance with the invention, the gene of the antibody is introduced into an expression vector such that the gene is expressed under the control of an expression-regulating region, for example enhancer and promoter. Then, a host cell is transformed with the expression vector, to express the antibody.

[0062] The gene of the antibody may be expressed by separately inserting DNA encoding the heavy chain (H chain) of the antibody and DNA encoding the light chain (L chain) thereof in expression vectors to simultaneously transform a host cell, or by inserting DNAs encoding the H chain and the L chain in a single expression vector to transform a host cell (see WO 94/11523).

[0063] Additionally, not only such host cells but also transgenic animal can be used for generating a recombinant antibody. For example, the gene of the antibody is inserted intermediately into a gene encoding a protein (e.g., goat β casein) generated inherently in milk to prepare a fusion gene. The DNA fragment comprising the fusion gene with the gene of the antibody as inserted therein is injected in a goat embryo, which is introduced in a female goat. The desired antibody is obtained from the milk produced by a transgenic goat born from the goat having received the embryo or a progeny thereof. So as to increase the amount of milk containing the desired antibody as produced by the transgenic goat, hormone may appropriately be administered to the transgenic goat (Ebert, K. M. et al., *Bio/Technology* (1994) 12, 699-702).

[0064] In accordance with the invention, artificially modified recombinant antibodies, for example a chimera antibody (e.g., humanized antibody) may also be used. These modified antibodies can be produced, using existing methods. In case that the antibody of the invention is to be used as an antibody for therapeutic treatment, the genetic recombinant type antibody is preferably used.

[0065] Chimera antibody can be obtained by linking the DNA encoding the V region of the antibody as obtained in the manner described above to DNA encoding the C region of a human antibody, inserting the resulting DNA in an expression vector, and introducing the vector in a host for production of the antibody. Using this existing method, a chimera antibody useful in accordance with the invention can be obtained.

[0066] Humanized antibody is also referred to as reshaped human antibody and is prepared by transplanting the complementarity determining region (CDR) of an antibody of mammals except humans, for example mouse, into the complementarity determining region of a human antibody. General genetic recombination techniques thereof are also known in the art (see European Patent Application EP 125023; WO 96/02576).

[0067] Specifically, a DNA sequence designed such that the CDR of mouse antibody can be linked to the framework region (FR) of human antibody is synthetically prepared by PCR, using several oligonucleotides prepared in such a manner that the oligonucleotides might have parts overlapped with the terminal regions of both CDR and FR (see the method described in WO 98/13388).

[0068] The FR region of human antibody to be linked to CDR is selected such that the CDR can form a good antigen binding site. If necessary, the amino acids in the FR in the V region of the antibody may be substituted, so that the CDR of the reshaped human antibody may form an appropriate antigen binding site (Sato, K. et al., *Cancer Res.* (1993) 53, 851-856).

[0069] As the C regions of chimera antibody and humanized antibody, those of human antibody are used; for example, C γ 1, C γ 2, C γ 3, and C γ 4 can be used for the H chain, while C κ and C λ can be used for the L chain. So as to improve the stability of the antibody or the production thereof, the C region of human antibody may be modified.

[0070] Preferably, the chimera antibody contains a sequence of an antibody derived from mammals except humans in the V region, and contains a sequence derived from a human antibody in the C region.

[0071] Humanized antibody comprises the CDR of an antibody derived from mammals except humans, and the FR and C regions derived from a human antibody. Because the antigenicity of chimera antibody such as humanized antibody is reduced in humans, chimera antibody is useful as an active component of a therapeutic agent of the invention.

[0072] The antibody for use in accordance with the invention is not only the whole antibody molecule but also a fragment of the antibody or a modified product thereof, including divalent antibody and monovalent antibody, as long as such fragment or such modified product can bind to the GPC3 N-terminal peptide or the GPC3 C-terminal peptide. For example, the antibody fragment includes Fab, F(ab')₂, Fv, Fab/C having one Fab and complete FC, or single chain Fv (scFv) where Fv of the H chain and the L chain are linked via an appropriate linker. Specifically, the antibody is treated with enzymes, for example papain and pepsin, to generate antibody fragments. Otherwise, genes encoding these antibody fragments are constructed, introduced in an expression vector and expressed in an appropriate host cell (see for example, Co, M. S. et al., *J. Immunol.* (1994) 152, 2968-2976; Better, M. & Horwitz, A. H. *Methods in Enzymology* (1989) 178, 476-496, Academic Press, Inc.; Plueckthun, A. & Skerra, A. *Methods in Enzymology* (1989) 178, 476-496, Academic Press, Inc.; Lamoyi, E., *Methods in Enzymology* (1989) 121, 652-663; Rousseaux, J. et al.,

Methods in Enzymology (1989) 121, 663-669; Bird, R. E. et al., TIBTECH (1991) 9, 132-137).

[0073] ScFv can be obtained by linking the V region of the H chain and the V region of the L chain of an antibody. In this scFv, the V region of the H chain and the V region of the L chain are linked together via a linker, preferably a peptide linker (Huston, J. S. et al., Proc. Natl. Acad. Sci. U.S.A. (1988) 85, 5879-5883). The V region of the H chain and the V region of the L chain in scFv may be derived from any antibodies described herein. Any appropriate single-stranded peptide comprising 12 to 19 amino acid residues may be used as the peptide linker for linking the V regions.

[0074] DNA encoding scFv is obtained by first amplifying DNA encoding the H chain or the V region of the H chain and the DNA encoding the L chain or the V region of the L chain by using as a template a portion of DNA encoding all the sequences thereof or a desired amino acid sequence therein and a pair of primers defining both the ends, and then amplifying the DNA with DNA encoding the peptide linker and a pair of primers defined in such a manner that both the ends of the peptide linker may be linked respectively to the H chain and the L chain.

[0075] Once the DNA encoding scFv is prepared, an expression vector carrying the DNA and a host transformed with the expression vector can be obtained by conventional methods. scFv can be obtained using the host by conventional methods.

[0076] The antibody fragments can be generated by obtaining and expressing the gene in the same manner as described above and allowing a host to produce the fragments. The "antibody" in accordance with the invention includes such antibody fragments.

[0077] There may also be used a modified product of the antibody, for example, anti-glypican antibodies conjugated with various molecules such as labeling substances, toxin, and radioactive materials. The "antibody" in accordance with the invention includes these modified antibodies. Such modified antibodies can be obtained by chemical modification of an antibody. Methods for modifying antibodies have already been established in the art.

[0078] Further, the antibody for use in accordance with the invention may be a bispecific antibody. The bispecific antibody may include those having antigen binding sites recognizing different epitopes on the N-terminal peptide of GPC3 or the C-terminal peptide of GPC3. Alternatively, one of the antigen binding sites recognizes the N-terminal peptide of GPC3 or the C-terminal peptide of GPC3, while the other antigen binding site may recognize a labeling substance and the like. Such bispecific antibody can be prepared or obtained by linking HL pairs of two types of antibodies or by fusing hybridomas generating different monoclonal antibodies together to prepare a fusion cell capable of producing a bispecific antibody. Further, such bispecific antibody can be prepared by genetic engineering technique.

[0079] In accordance with the invention, an antibody with a modified sugar chain may also be used for the purpose of enhancing cytotoxic activity. Modification technique of the sugar chain of antibody is known in the art (for example, WO 00/61739, WO 02/31140, etc.).

[0080] The antibody gene constructed in the manner described above can be expressed and obtained by known methods. In case of a mammalian cell, a conventional useful promoter, the antibody gene to be expressed and poly (A) signal downstream the 3' side thereof are functionally linked for the expression. For example, the promoter/enhancer includes human cytomegalovirus immediate early promoter/enhancer.

[0081] Additionally, the promoter/enhancer for use in the expression of the antibody for use in accordance with the invention includes, for example, virus promoters including retrovirus, polyoma virus, adenovirus and simian virus 40 (SV40)/enhancer or promoters derived from mammalian cells such as human elongation factor 1a (HEF1a)/enhancer.

[0082] In case of using SV40 promoter/enhancer, gene expression can readily be done by the method of Mulligan et al. (Nature (1979) 277, 108). In case of using the HEF1a promoter/enhancer, gene expression can readily be done by the method of Mizushima et al. (Nucleic Acids Res. (1990) 18, 5322).

[0083] In case of Escherichia coli, a useful conventional promoter, a signal sequence for antibody secretion and an antibody gene to be expressed are functionally linked for expressing the gene. The promoter includes for example lacZ promoter and araB promoter. In case that lacZ promoter is to be used, the gene can be expressed by the method of Ward et al. (Nature (1998), 341, 544-546; FASEB J. (1992) 6, 2422-2427). In case that araB promoter is to be used, the gene can be expressed by the method of Better et al. (Science (1988) 240, 1041-1043).

[0084] As the signal sequence for antibody secretion, pelB signal sequence (Lei, S. P. et al. J. Bacteriol. (1987) 169, 4379) may be used when the antibody is generated in the periplasm of Escherichia coli. After the antibody generated in the periplasm is separated, the structure of the antibody is appropriately refolded for use.

[0085] As the replication origin, those from SV40, polyoma virus, adenovirus and bovine papilloma virus (BPV) may be used. For amplification of the copy number of the gene in a host cell system, the expression vector may carry a selective marker, for example, aminoglycoside transferase (APH) gene, thymidine kinase (TK) gene, Escherichia coli xanthine guanine phosphoribosyl transferase (Ecogpt) gene and dehydrofolate reductase (dhfr) gene.

[0086] So as to produce the antibody for use in accordance with the invention, an appropriate expression system, for example eukaryotic cell or prokaryotic cell system can be used. The eukaryotic cell includes for example established animal cell lines such as mammalian cell lines, insect cell lines, fungal cells and yeast cells. The prokaryotic cell includes for example bacterial cells such as Escherichia coli cell.

[0087] Preferably, the antibody for use in accordance with the invention is expressed in mammalian cells, for example

CHO, COS, myeloma, BHK, Vero, and HeLa cell.

[0088] The transformed host cell is cultured in vitro or in vivo to produce the intended antibody. The host cell may be cultured by known methods. As the culture medium, for example, DMEM, MEM, RPMI 1640 and IMDM can be used. Auxiliary serum fluid such as fetal calf serum (FCS) may also be used in combination.

[0089] The antibody expressed and generated as described above can be separated from such cells or host animals and can then be purified to homogeneity. The antibody for use in accordance with the invention can be separated and purified using an affinity column. A protein A column includes, for example, Hyper D, POROS, Sepharose F. F. (manufactured by Pharmacia). Additionally, any separation and purification methods generally used for protein may be employed in the invention. For example, chromatography columns other than affinity column, filter, ultrafiltration, salting-out, and dialysis may be used in combination to separate and purify the antibody (Antibodies A Laboratory Manual, Ed. Harlow, David Lane, Cold Spring Harbor Laboratory, 1988).

2. Detection of GPC3

[0090] Using the antibody against the N-terminal peptide of GPC3 in accordance with the invention, GPC3 in a test sample can be detected.

[0091] GPC3 to be detected using the antibody of the invention includes, but is not limited to, full-length GPC3 and fragments thereof. So as to detect GPC3 fragments, preferably, a fragment of the N-terminal peptide is detected.

[0092] The method for detecting the GPC3 protein in a test sample is not specifically limited. The GPC3 protein is preferably detected by an immunoassay method using the anti-GPC3 N-terminal peptide antibody. The immunoassay method includes, for example, radioimmunoassay, enzyme immunoassay, fluorescent immunoassay, luminescent immunoassay, immunoprecipitation method, immunonephelometry, western blot technique, immunostaining, and immunodiffusion method. Preferably, the immunoassay method is enzyme immunoassay. Particularly preferably, the immunoassay method is enzyme-linked immunosorbent assay (ELISA) (for example, sandwich ELISA). The immunoassay method such as ELISA as described above can be done by a person skilled in the art according to known methods.

[0093] General detection methods using the anti-GPC3 N-terminal peptide antibody to detect the GPC3 protein in a test sample involve, for example, immobilizing the anti-GPC3 N-terminal peptide antibody on a support, adding a test sample to the support for incubation to bind the GPC3 protein to the anti-GPC3 N-terminal peptide antibody, rinsing the support and detecting the GPC3 protein bound through the anti-GPC3 N-terminal peptide antibody to the support.

[0094] The support for use in accordance with the invention includes, for example, insoluble polysaccharides such as agarose and cellulose, synthetic resins such as silicone resin, polystyrene resin, polyacrylamide resin, nylon resin and polycarbonate resin, and insoluble supports such as glass. These supports can be used in the forms of beads and plates. In case of beads, a column packed with beads can be used. In case of plates, multi-well plate (for example, 96-well multi-well plate) and biosensor chip can be used. The anti-GPC3 N-terminal peptide antibody can be bound to the support by general methods such as chemical binding and physical adsorption. Such supports are commercially available.

[0095] The binding of the anti-GPC3 N-terminal peptide antibody to the GPC3 protein is generally done in buffers. For example, phosphate buffer, Tris buffer, citric acid buffer, borate salt buffer, and carbonate salt buffer may be used as a buffer. Incubation may be carried out under conditions commonly used, for example, 4 °C to ambient temperature for one hour to 24 hours. Rinsing after incubation may be done using any solutions which do not inhibit the binding of the GPC3 protein to the anti-GPC3 N-terminal peptide antibody. For example, buffers containing surfactants such as Tween 20 may be used.

[0096] For the method for detecting the GPC3 protein in accordance with the invention, a control sample may be placed in addition to a test sample containing GPC3 protein to be detected. The control sample includes, for example, a negative control sample containing no GPC3 protein or a positive control sample containing the GPC3 protein. In this case, the GPC3 protein in the test sample can be detected by comparison with the results obtained using the negative control sample containing no GPC3 protein and the results obtained using the positive control sample containing the GPC3 protein. Additionally, a series of control samples having serially varied concentrations are prepared and the results of detection in the individual control samples are obtained in numerical figure to prepare a standard curve. Based on the standard curve, the GPC3 protein contained in the test sample can be determined quantitatively, based on the numerical figure about the test sample.

[0097] A preferable embodiment of the detection of the GPC3 protein bound through the anti-GPC3 N-terminal peptide antibody to the support includes a method using the anti-GPC3 N-terminal peptide antibody labeled with a labeling substance.

[0098] For example, a test sample is put in contact with the anti-GPC3 antibody immobilized on a support, which is then rinsed, to detect the GPC3 protein using a labeled antibody specifically recognizing the GPC3 protein.

[0099] In this case, the anti-GPC3 N-terminal peptide antibody immobilized on the support and anti-GPC3 N-terminal

peptide C antibody labeled with a labeling substance may recognize the same epitope of the GPC3 molecule, but preferably recognize different epitopes.

[0100] The anti-GPC3 N-terminal peptide antibody can be labeled by generally known methods. Any labeling substances known to a person skilled in the art can be used, including for example fluorescent dye, enzyme, coenzyme, chemiluminescent substance and radioactive substance. Specific examples thereof include for example radioisotopes (^{32}P , ^{14}C , ^{125}I , ^3H and ^{131}I), fluorescein, rhodamine, dansylchloride, umbelliferone, luciferase, peroxidase, alkaline phosphatase, β -galactosidase, β -glucosidase, horse radish peroxidase, glucoamylase, lysozyme, saccharide oxidase, microperoxidase, and biotin. Preferably, in the case that biotin is used as a labeling substance, avidin bound with enzymes such as alkaline phosphatase is further added after the addition of a biotin-labeled antibody. For binding the anti-GPC3 antibody with a labeling substance, any of the known methods such as glutaraldehyde method, maleimide method, pyridyl disulfide method and periodate method may be used.

[0101] Specifically, a solution containing the anti-GPC3 N-terminal peptide antibody is added to a support, such as a plate, to immobilize anti-GPC3 N-terminal peptide antibody. After rinsing the plate, the plate is blocked with for example BSA, so as to prevent non-specific protein binding. After rinsing again, a test sample is added to the plate. After incubation, the plate is rinsed, to which the labeled anti-GPC3 antibody is added. After appropriate incubation, the plate is rinsed and the labeled anti-GPC3 antibody remaining on the plate is detected. The detection can be done by methods known to a person skilled in the art. For example, in case of labeling with a radioactive substance, the detection can be done by a liquid scintillation or a RIA method. In case of labeling with an enzyme, a substrate for the respective enzyme is added to detect enzymatic substrate changes via for example color development by spectrophotometer. Specific examples of such substrate include 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid)diammonium salt (ABTS), 1,2-phenylenediamine (ortho-phenylenediamine), and 3,3',5,5'-tetramethylbenzidine (TME). In case of labeling with a fluorescent substance, the fluorescent substance can be detected with fluorophotometer.

[0102] A particularly preferable embodiment of the method for detecting the GPC3 protein in accordance with the invention involves using anti-GPC3 N-terminal peptide antibody labeled with biotin and avidin. Specifically, a solution containing anti-GPC3 N-terminal peptide antibody is added to a support such as plate, to immobilize the anti-GPC3 N-terminal peptide antibody. After rinsing the plate, the antibody is blocked with for example BSA to prevent non-specific protein binding. After rinsing again, a test sample is added to the plate. After incubation, the plate is rinsed, and the biotin-labeled anti-GPC3 antibody is added. After appropriate incubation, the plate is rinsed, and avidin conjugated to an enzyme, such as alkaline phosphatase or peroxidase is added. After incubation, the plate is rinsed, a substrate corresponding to each enzyme conjugated to avidin is added, and the GPC3 protein is detected using an enzymatic substrate change as an indicator.

[0103] Another embodiment of the method for detecting the GPC3 protein in accordance with the invention involves using a primary antibody specifically recognizing the GPC3 protein and a secondary antibody specifically recognizing the primary antibody.

[0104] For example, a test sample is put in contact with the anti-GPC3 N-terminal peptide antibody immobilized on a support. After incubation, the support is rinsed and the GPC3 protein bound to the support after rinsing is detected using a primary anti-GPC3 antibody and a secondary antibody specifically recognizing the primary antibody. In this case, the secondary antibody is preferably labeled with a labeling substance.

[0105] Specifically, a solution containing anti-GPC3 N-terminal peptide antibody is added to a support, such as plate, to immobilize the anti-GPC3 N-terminal peptide antibody. After rinsing the plate, the antibody is blocked with for example BSA to prevent non-specific protein binding. After rinsing again, a test sample is added to the plate. After incubation, the plate is rinsed and a primary anti-GPC3 antibody is added. After appropriate incubation, the plate is rinsed and a secondary antibody specifically recognizing the primary antibody is added. After appropriate incubation, the plate is rinsed and the secondary antibody remaining on the plate is detected. The detection of the secondary antibody can be done by the methods described above.

[0106] Still another embodiment of the method for detecting the GPC3 protein in accordance with the invention involves using an aggregation reaction. In this method, GPC3 can be detected using a carrier sensitized with the anti-GPC3 N-terminal peptide antibody. Any carriers may be used as the carrier to be sensitized with the antibody, as far as the carrier is insoluble and stable and does not undergo non-specific reaction. For example, latex particle, bentonite, collodion, kaolin and immobilized sheep erythrocyte may be used. Latex particle is preferably used. Latex particles include, for example, polystyrene latex particle, styrene-butadiene copolymer latex particle, and polyvinyltoluene latex particle. Polystyrene latex particle is preferably used. After the sensitized particle is mixed with a sample and agitated for a given period of time, GPC3 can be detected by observing the aggregation under naked eyes since the aggregation level of such particle is higher as the GPC3 antibody is contained at a higher concentration in the sample. Additionally, the turbidity due to the aggregation can be measured with spectrophotometer and the like, to detect GPC3.

[0107] Another embodiment of the method for detecting the GPC3 protein in accordance with the invention involves using a biosensor utilizing surface plasmon resonance phenomenon. The biosensor utilizing surface plasmon resonance phenomenon enables the observation of the protein-protein interaction as surface plasmon resonance signal

on real time using a trace amount of protein without labeling. For example, the binding of the GPC3 protein to the anti-GPC3 N-terminal peptide antibody can be detected by using biosensors such as BIAcore (manufactured by Pharmacia). Specifically, a test sample is put in contact with a sensor chip having the anti-GPC3 N-terminal peptide antibody immobilized thereon, and the GPC3 protein bound to the anti-GPC3 N-terminal peptide antibody is detected as the change of the resonance signal.

[0108] The detection methods in accordance with the invention may be automated using various automatic laboratory apparatuses, so that a large volume of samples can be tested at a time.

[0109] It is an objective of the invention to provide a diagnostic reagent or kit for detecting GPC3 protein in a test sample for cancer diagnosis. The diagnostic reagent or kit contains at least the anti-GPC3 N-terminal peptide antibody. In case that the diagnostic reagent or kit is based on EIA, a carrier for immobilizing the antibody may be contained, or the antibody may be preliminarily bound to a carrier. In case that the diagnostic reagent or kit is based on the aggregation method using carriers such as latex, the reagent of kit may contain a carrier having the antibody adsorbed thereon. Additionally, the kit may appropriately contain, for example, a blocking solution, a reaction solution, a reaction-terminating solution and reagents for treating sample.

3. Disruption of cancer cell using the anti-GPC3 C-terminal peptide antibody and cancer therapy using the same

(1) Determination of antibody activity

[0110] The antigen binding activity of the antibody for use in accordance with the invention may be assayed using known techniques (Antibodies A Laboratory Manual. Ed. Harlow, David Lane, Cold Spring Harbor Laboratory, 1988) and an activity of inhibiting the ligand-receptor binding thereof (Harada, A. et al., International Immunology (1993) 5, 681-690).

[0111] A method for assaying the antigen binding activity of the anti-GPC3 C-terminal peptide antibody for use in accordance with the invention includes ELISA (enzyme-linked immunosorbent assay), EIA (enzyme immunoassay), RIA (radioimmunoassay) and fluorescent antibody method. In enzyme immunoassay, a sample containing the anti-GPC3 C-terminal peptide antibody, for example a culture supernatant of a cell producing the anti-GPC3 C-terminal peptide antibody or the purified antibody is added to a plate coated with the GPC3 C-terminal peptide. A secondary antibody labeled with an enzyme such as alkali phosphatase is added and the plate is incubated and rinsed, then an enzyme substrate such as p-nitrophenylphosphoric acid is added to measure the absorbance and assess the antigen binding activity.

[0112] So as to determine the activity of the antibody for use in accordance with the invention, the neutralization activity of the anti-GPC3 C-terminal peptide antibody is measured.

(2) Cytotoxicity

[0113] For therapeutic purpose, the antibody for use in accordance with the invention preferably has the ADCC activity or the CDC activity as cytotoxicity.

[0114] The ADCC activity can be assayed by mixing an effector cell, a target cell and the anti-GPC3 C-terminal peptide antibody together and examining the ADCC level. As the effector cell, cell such as mouse splenocyte and mononuclear cell separated from human peripheral blood or bone marrow can be utilized. As the target cell, a human cell line such as human hepatoma line HuH-7 can be used. The target cells are preliminarily labeled with ⁵¹Cr and incubated with the anti-GPC3 C-terminal peptide antibody, then effector cells at an appropriate ratio is added to the target cells and incubated. After incubation, the supernatant is collected to count the radioactivity in the supernatant, to assay the ADCC activity.

[0115] Further, the CDC activity can be assayed by mixing the labeled target cell described above with the anti-GPC3 C-terminal peptide antibody, subsequently adding complement, and counting the radioactivity in the supernatant after incubation.

[0116] The Fc moiety is needed for the antibody to exert the cytotoxicity. In case that the inhibitor of cell proliferation in accordance with the invention utilizes the cytotoxicity of the antibody, thus, the anti-GPC3 C-terminal peptide antibody for use in accordance with the invention preferably contains the Fc moiety.

(3) Cell disruption

[0117] The anti-GPC3 C-terminal peptide antibody of the invention may also be used for cell disruption, particularly the disruption of cancer cell. Further, the anti-GPC3 C-terminal peptide antibody of the invention can be used as an anticancer agent. Cancers to be therapeutically treated and prevented by the antibody of the invention include, but are not limited to, hepatoma, lung cancer, colon cancer, breast cancer, prostate cancer, pancreatic cancer and lymphoma,

preferably Hepatoma.

(4) Administration method and pharmaceutical formulation

5 [0118] The cell disrupting agent or anticancer agent in accordance with the invention is used for the purpose of therapeutically treating or ameliorating diseases caused by abnormal cell growth, particularly cancer.

[0119] The effective dose is selected within a range of 0.001 mg to 1,000 mg per 1 kg body weight. Also the effective dose is selected within a range of 0.01 mg to 100,000 mg/body weight per patient. However, the dose of the therapeutic agents containing the anti-GPC3 C-terminal peptide antibody of the invention are not limited to the above doses.

10 [0120] The timing for administering the therapeutic agent of the invention is either before or after the onset of clinical symptoms of the diseases.

[0121] The therapeutic agent comprising the anti-GPC3 C-terminal-peptide antibody in accordance with the invention as an active component can be formulated by a conventional method (Remington's Pharmaceutical Science, latest edition, Mark Publishing Company, Easton, USA), and may also contain pharmaceutically acceptable carriers and additives.

15 [0122] Examples of such carriers and pharmaceutical additives include water, pharmaceutically acceptable organic solvents, collagen, polyvinyl alcohol, polyvinyl pyrrolidone, carboxyvinyl polymer, carboxymethyl cellulose sodium, polyacrylate sodium, sodium alginate, water-soluble dextran, carboxymethyl starch sodium, pectin, methyl cellulose, ethyl cellulose, gum xanthan, gum arabic, casein, agar, polyethylene glycol, diglycerin, glycerin, propylene glycol, vaseline, paraffin, stearyl alcohol, stearic acid, human serum albumin (HSA), mannitol, sorbitol, lactose and surfactants acceptable as pharmaceutical additives.

20 [0123] In practice, an additive or a combination thereof is selected depending on the dosage form of the therapeutic agent of the invention. However, the additive is not limited to those described above. In case that the therapeutic agent is to be used in an injection formulation, the purified anti-GPC3 C-terminal peptide antibody of the invention is dissolved in a solvent, such as physiological saline, buffers, and glucose solution, and adsorption preventing agents such as Tween 80, Tween 20, gelatin and human serum albumin is added. Alternatively, the therapeutic agent is provided in a freeze-dried form as a dosage form to be dissolved and reconstituted prior to use. As excipients for freeze-drying, for example, sugar alcohols such as mannitol and glucose and sugars may be used.

30 Brief Description of the Drawings

[0124]

35 Fig. 1 shows bar graphs depicting the results of the analysis of GPC3 mRNA expression using Gene Chip, where Fig. 1A depicts GPC3 expression and Fig. 1B depicts the expression of alpha-fetoprotein (AFP). NL, CH, LC, WD, MD and PD on the horizontal axis represent normal liver, inflammatory lesion of hepatitis, lesion of liver cirrhosis, well-differentiated cancer, moderately differentiated cancer and poorly differentiated cancer, respectively.

Fig. 2 shows images of purified soluble GPC3 of heparan sulfate adduct type and the GPC3 core protein, as stained with CBB.

40 Fig. 3 shows bar graphs depicting the expression of the GPC3 gene in human hepatoma.

Fig. 4 shows the results of western blotting of the soluble form of the core protein using the anti-GPC3 antibody.

Fig. 5 shows the principle of sandwich ELISA using the anti-GPC3 antibody.

Fig. 6 is a graph of the standard curve for the GPC3 sandwich ELISA using M6B1 and M18D4.

Fig. 7 is a schematic view of the GPC3 structure.

45 Fig. 8 shows combinations of the anti-GPC3 antibodies employed in ELISA.

Fig. 9 is a graph of the standard curve for the GPC3 sandwich ELISA system using various combinations of the anti-GPC3 antibodies.

Fig. 10 shows the assay results of the ADCC activity of the anti-GPC3 antibody.

Fig. 11 shows the assay results of the CDC activity of the anti-GPC3 antibody.

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Best Mode for Carrying out the Invention

[0125] The invention is now specifically described in the following Examples. However, the invention is not limited by the Examples.

55 [0126] In the Examples described in this specification, the following materials were used.

[0127] As expression vectors of the soluble form of GPC3 and the soluble form of the GPC3 core protein, pCXND2 and pCXND3 prepared by integrating the DHFR gene and the neomycin-resistant gene in pCAGGS were used.

[0128] DXB11 was purchased from ATCC. For culturing, 5 % FBS (GIBCO BRL CAT# 10099-141, Lot#

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AO275242/Minimum Essential Medium Alpha medium (α MEM (+)) (GIBCO BRL CAT# 12571-071)/1 % Penicillin-Streptomycin (GIBCO BRL CAT# 15140-122) was used. For selection of stable cell line of DXB11 expressing each protein, 500 μ g/mL Geneticin (GIBCO BRL CAT# 10131-027)/5 % FBS/ α MEM without ribonucleotides and deoxyribonucleotides (GIBCO BRL CAT# 12561-056)(α MEM(-))/PS was used alone or with supplemented with MTX to a final concentration of 25 nM.

[0129] HepG2 was purchased from ATCC and maintained in 10 % FBS/Dulbecco's modified Eagle medium (DMEM) (GIBCO BRL. CAT# 11995-065)/PS.

[0130] The hybridoma was maintained in 10 % FBS/RPMI1640/1 \times HAT media supplement (SIGMA CAT# H-0262) /0.5 \times BM-Condensed H1 Hybridoma cloning supplement (Roche CAT# 1088947).

Example 1

Cloning and expression analysis of human GPC3 (GPC3) cDNA Cloning of full-length cDNA encoding human glypican 3 (GPC3 hereinafter)

[0131] The full-length cDNA encoding human GPC3 was amplified by PCR, using as a template a first strand cDNA prepared from a colon cancer cell line Caco2 by a general method and Advantage 2 kit (Clontech Cat. No. 8430-1). Specifically, 50 μ l of a reaction solution containing Caco2-derived cDNA of 2 μ l, 1 μ l of a sense primer (SEQ ID NO: 1), 1 μ l of an antisense primer (SEQ ID NO: 2), 5 μ l of Advantage2 10 \times PCR buffer, 8 μ l of dNTP mix (1.25 mM) and 1.0 μ l of Advantage polymerase Mix was subjected to 35 cycles of 94 $^{\circ}$ C for one minute, 63 $^{\circ}$ C for 30 seconds and 68 $^{\circ}$ C for 3 minutes. The amplified product from the PCR (inserted in TA vector pGEM-T easy using pGEM-T Easy Vector System I (Promega Cat No. A1360)) was sequenced using ABI3100 DNA sequencer to confirm that cDNA encoding the full-length human GPC3 was isolated. The sequence represented by SEQ ID NO: 3 indicates the nucleotide sequence of the human GPC3 gene, while the sequence represented by SEQ ID NO: 4 indicates the amino acid sequence of human GPC3 protein.

SEQ ID NO: 1: GATATC-ATGGCCGGGACCGTGCGCACCGCGT

SEQ ID NO: 2: GCTAGC-TCAGTGCACCAGGAAGAAGAAGCAC

Expression Analysis of human GPC3 mRNA using GeneChip

[0132] mRNA expression was analyzed in 24 cases with hepatoma lesions (well-differentiated cancer: WD; moderately differentiated cancer: MD; poorly differentiated cancer: PD), 16 hepatoma cases with non-cancer lesions (hepatitis lesion: CH, cirrhosis lesion: LC), 8 cases with normal liver: NL (informed consent acquired; available from Tokyo University, School of Medicine and Saitama Cancer Center), using GeneChipTM UG-95A Target (Affymetrix). Specifically, total RNA was prepared using ISOGEN (Nippon Gene) from the individual tissues, from which 15 μ g each of total RNA was used for gene expression analysis according to the Expression Analysis Technical Manual (Affymetrix).

[0133] As shown in Fig. 1, the mRNA expression level of human GPC3 gene (Probe Set ID: 39350_at) was apparently higher in many of the cases compared with the expression in normal liver tissue, despite the differentiation stages of hepatoma. Furthermore, comparison was made with the mRNA expression of alpha-fetoprotein (Probe Set ID: 40114_at) most commonly used as a diagnostic marker of hepatoma currently. It was shown that even in well-differentiated cancer showing almost no such mRNA expression of alpha-fetoprotein, sufficiently enhanced mRNA expression of GPC3 was observed, and that the ratio of the activation of the mRNA expression of GPC3 was higher. Thus, it is considered that GPC3 detection is useful as a diagnostic method of hepatoma at an early stage.

Example 2

Preparation of anti-GPC3 antibody

Preparation of the soluble form of human GPC3

[0134] As a material for preparing anti-GPC3 antibody, the soluble form of the GPC3 protein lacking the hydrophobic region on the C-terminal side was prepared.

[0135] Using a plasmid DNA containing the complete full-length human GPC3 cDNA supplied from Tokyo University,

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Advanced Technology Institute, a plasmid DNA for expressing the soluble form of the GPC3 cDNA was constructed. PCR was conducted using a downstream primer (5'-ATA GAA TTC CAC CAT GGC CGG GAC CGT GCG C-3') (SEQ ID NO: 5) designed to remove the hydrophobic region on the C-terminal side (564-580 amino acid), and an upstream primer (5'-ATA GGA TCC CTT CAG CGG GGA ATG AAC GTT C-3') (SEQ ID NO:6) with the EcoRI recognition sequence and the Kozak's sequence having been added. The resulting PCR fragment (1711 bp) was cloned in pCXND2-Flag. The prepared expression plasmid DNA was introduced in a CHO cell line DXB11. Selection with 500 µg/mL Geneticin resulted in a CHO line highly expressing the soluble form of GPC3.

[0136] Using a 1700-cm² roller bottle, the CHO line highly expressing the soluble form of GPC3 was cultured at a large scale, and the culture supernatant was collected for purification. The culture supernatant was applied to DEAE Sepharose Fast Flow (Amersham CAT# 17-0709-01), washed, and eluted with a buffer containing 500 mM NaCl. Subsequently, the product was affinity purified using Anti-Flag M2 agarose affinity gel (SIGMA CAT# A-2220) and eluted with 200 µg/mL Flag peptide. After concentration with Centriprep-10 (Millipore Cat# 4304), the Flag peptide was removed by gel filtration with Superdex 200 HR 10/30 (Amersham CAT# 17-1088-01). Finally, the product was concentrated using DEAE Sepharose Fast Flow column, and eluted with PBS (containing 500 mM NaCl) containing no Tween 20 for replacement of the buffer.

Preparation of the soluble form of human GPC3 core protein

[0137] Using the wild type human GPC3 cDNA as template, cDNA was prepared by assembly PCR, where Ser 495 and Ser 509 were substituted with Ala. A primer was designed in such a fashion that His tag might be added to the C terminus. The resulting cDNA was cloned in pCXND3 vector. The prepared expression plasmid DNA was introduced in a DXB11 line, followed by selection with 500 µg/mL Geneticin, to obtain the CHO line highly expressing the soluble form of the GPC3 core protein.

[0138] A large scale cultivation was done with a 1700-cm² roller bottle, and the culture supernatant was collected for purification. The supernatant was applied to Q sepharose Fast Flow (Amersham CAT# 17-0510-01), washed, and eluted with a phosphate buffer containing 500 mM NaCl. Subsequently, the product was affinity purified using Chelating Sepharose Fast Flow (Amersham CAT# 17-0575-01), and eluted with a gradient of 10-150 mM imidazole. Finally, the product was concentrated with Q sepharose Fast Flow and eluted with a phosphate buffer containing 500 mM NaCl.

[0139] SDS polyacrylamide gel electrophoresis showed a smear-like band of 50 to 300 kDa and a band of about 40 kDa. Fig.2 shows the results of the electrophoresis. GPC3 is a proteoglycan of 69 kDa and with a heparan sulfate-addition sequence at the C terminus. It was considered that the smear-like band corresponds to GPC3 modified with heparan sulfate. The results of amino acid sequencing indicated that the band of about 40 kDa had an origin in the N-terminal fragment. Thus, it was anticipated that GPC3 was more or less cleaved.

[0140] So as to remove antibodies against heparan sulfate in the following screening for hybridoma, the soluble form of the GPC3 core protein where a heparan sulfate-addition signal sequence Ser 495 and Ser 509 were substituted with Ala. CHO cell line highly expressing the protein was prepared as above, and the culture supernatant was affinity purified utilizing the His-tag. SDS polyacrylamide gel electrophoresis showed three bands of 70 kDa, 40 kDa and 30 kDa. Amino acid sequencing indicated that the band of 30 kDa was the C-terminal fragment of GPC3. The C-terminal fragment starts from serine 359 or from valine 375. Thus, it was anticipated that GPC3 received some enzymatic cleavage. The reason why the band of 30 kDa was not observed in the GPC3 of heparan sulfate-added type was that the fragment formed the smear-like band due to the addition of heparan sulfate. It is a novel finding that GPC3 receives enzymatic cleavage at a specific amino acid sequence, but the biological meaning thereof has not yet been elucidated.

[0141] The inventors made an assumption on the basis of the results that GPC3 on the membrane even in hepatoma patients would be cleaved and secreted as the soluble form in blood. Compared with AFP as a hepatoma marker, the expression of the gene of GPC3 was found higher in hepatoma patients at earlier stages (Fig. 1). So as to examine the possibility as a novel tumor marker with higher clinical utility than that of AFP, an anti-GPC3 antibody was prepared to construct a sandwich ELISA system as described in Example 2 or below.

Preparation of anti-GPC3 antibody

[0142] Because the homology of human GPC3 with mouse GPC3 is as high as 94 % at the amino acid levels, it was considered that it might be difficult to obtain the anti-GPC3 antibody by the immunization of normal mouse with human GPC3. Thus, MRL/lpr mouse with autoimmune disease was used as an animal to be immunized. Five MRL/lpr mice (CRL) were immunized with the soluble form of GPC3. For the first immunization, the immunogen protein was adjusted to 100 µg/animal and was then emulsified using FCA (Freund's complete adjuvant (H37 Ra), Difco (3113-60), Becton Dickinson (cat# 231131)), which was then subcutaneously administered to the mice. Two weeks later, the protein was adjusted to 50 µg/animal and emulsified with FIA (Freund's incomplete adjuvant, Difco (0639-60), Becton Dickinson (cat# 263910)) for subcutaneous administration to the mice. At one week interval since then, booster was carried out

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in total of 5 times. For final booster, the protein was diluted with PBS to 50 µg/animal, which was administered in the caudal vein. By ELISA using an immunoplate coated with the GPC3 core protein, it was confirmed that the serum antibody titer against GPC3 was saturated. A mouse myeloma cell P3U1 and mouse splenocyte were mixed together to allow for cell fusion in the presence of PEG1500 (Roche Diagnostics, cat# 783641). The resulting mixture was inoculated in a 96-well culture plate. From the next day, hybridoma was selected with the HAT medium, the culture supernatant was screened by ELISA. Positive clones were subjected to monocloning by limited dilution method. The resulted monoclonal was cultured at an enlarged scale and the culture supernatant was collected. The screening by ELISA was done using the binding activity to the GPC3 core protein as a marker to obtain six clones of an anti-GPC3 antibody with a strong binding potency.

[0143] The antibody was purified using Hi Trap Protein G HP (Amersham CAT# 17-0404-01). The supernatant from the hybridoma culture was applied directly to a column, washed with a binding buffer (20 mM sodium phosphate, pH 7.0) and eluted with an elution buffer (0.1 M glycine-HCl, pH 2.7). The eluate was collected into a tube containing a neutralization buffer (1 M Tris-HCl, pH 9.0) for immediate neutralization. After antibody fractions were pooled, the resulting pool was dialyzed against 0.05 % Tween 20/PBS overnight and for a whole day for buffer replacement. NaN₃ was added to the purified antibody to 0.02 %. The antibody was stored at 4 °C.

Analysis of anti-GPC3 antibody

[0144] The antibody concentration was assayed by mouse IgG sandwich ELISA using goat anti-mouse IgG (gamma) (ZYMED CAT# 62-6600) and alkali phosphatase-goat anti-mouse IgG (gamma) (ZYMED CAT# 62-6622), along with a commercially available purified mouse IgG1 antibody (ZYMED CAT# 02-6100) as a standard.

[0145] The isotyping of the anti-GPC3 antibody was done with ImmunoPure Monoclonal Antibody Isotyping Kit II (PIERCE CAT# 37502) by the method according to the attached manual. The results of the isotyping indicated that all of the antibodies were of IgG1 type.

[0146] By western blotting using the GPC3 core protein, the epitopes of the anti-GPC3 antibody were classified. The soluble form of the GPC3 core protein was applied to 10 % SDS-PAGE mini (TEFCO CAT# 01-075) at 100 ng/lane for electrophoresis (60 V for 30 min; 120 V for 90 min), and subsequently transferred on Immobilon-P (Millipore CAT# IPVH R85 10) using Trans-Blot SD Semi-Dry Electrophoretic Transfer Cell (BIO-RAD) (15 V for 60 min). After the membrane was gently rinsed with TBS-T (0.05 % Tween 20, TBS), the membrane was shaken with 5 % skim milk-containing TBS-T for one hour (at ambient temperature) or overnight (at 4 °C). After shaking with TBS-T for about 10 minutes, each anti-GPC3 antibody diluted with 1 % skim milk-containing TBS-T to 0.1 to 10 µg/ml was added for one-hour with shaking. The membrane was rinsed with TBS-T (10 minutes × three times) and shaken with HRP-anti-mouse IgG antibody (Amersham CAT# NA 931) diluted to 1.1000 with 1 % skim milk-containing TBS-T for one hour, and rinsed with TBS-T (10 minutes × three times). ECL-Plus (Amersham RPN 2132) was used for chromogenic reaction. Hyperfilm ECL (Amersham CAT# RPN 2103K) was used for detection. Fig. 4 shows the results of the western blotting analysis. For the classification, it was determined that the antibody reacting with the band of 40 kDa has an epitope at the N terminus, while the antibody reacting with the band of 30 kDa has an epitope at the C terminus. As antibodies recognizing the N-terminal side, M6B1, M18D4, and M19B11 were obtained. As antibodies recognizing the C-terminal side, M3C11, M13B3, and M3B8 were obtained. The results of the analysis using BIACORE indicated that the KD values of the individual antibodies were in the range of from 0.2 to 17.6 nM.

Example 3

Detection of the secreted form of GPC3

Mouse xenograft model

[0147] 3,000,000 human hepatoma HepG2 cells were transplanted under the abdominal skin in 6-weeks female SCID mice (Fox CHASE C. B-17/lcr-scidJcl, JapanClair) and nudemice (BALB/cAJcl-nu, Japan Clair). 53 days later when tumor was sufficiently formed, whole blood was drawn out from the posterior cava of HepG2-transplanted SCID mice #1, 3, and 4. Plasma was prepared in the presence of EDTA-2Na and aprotinin (Nipro Neotube vacuum blood tube, NIPRO, NT-EA0205) and stored at -20 °C until assay date. In the case of the HepG2-transplanted SCID mouse #2, whole blood was taken 62 days after HepG2 transplantation. In the case of the HepG2-transplanted nude mice #1 and #2, whole blood was taken 66 days after HepG2 transplantation. As a control, plasma was prepared from normal SCID mouse of the same age by the same procedures.

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Sandwich ELISA

[0148] So as to detect the secreted form of GPC3 in blood, a sandwich ELISA system of GPC3 was constructed. M6B1 was used as an antibody to be coated in a 96-well plate. M18D4 labeled with biotin was used as an antibody
5 detecting GPC3 bound to M6B1. For chromogenic reaction, AMPAK of DAKO was used for achieving high detection sensitivity.

[0149] A 96-well immunoplate was coated with the anti-GPC3 antibody diluted with a coating buffer (0.1 M NaHCO₃, pH 9.6, 0.02 w/v % NaN₃) to obtain a concentration of 10 µg/mL, and incubated at 4 °C overnight. On the next day, the plate was rinsed three times with 300 µl/well of rinse buffer (0.05 v/v %, Tween 20, PBS) and 200 µl of dilution
10 buffer (50 mM Tris-HCl, pH 8.1, 1 mM MgCl₂, 150 mM NaCl, 0.05 v/v % Tween 20, 0.02 w/v % NaN₃, 1 w/v % BSA) was added for blocking. After storage for several hours at ambient temperature or at 4 °C overnight, mouse plasma or the culture supernatant appropriately diluted with a dilution buffer was added and incubated at ambient temperature for one hour. After rinsing with RB at 300 µl/well three times, the biotin-labeled anti-GPC3 antibody diluted with a dilution
15 buffer to 10 µg/mL was added, and incubated at ambient temperature for one hour. After rinsing with RB at 300 µl/well three times, AP-streptoavidin (ZYMED) diluted to 1/1000 with a dilution buffer was added, and incubated at ambient temperature for one hour. After rinsing with the rinse buffer at 300 µl/well five times, AMPAK (DAKO CAT# K6200) was added for chromogenic reaction according to the attached protocol, and the absorbance was measured with a micro-plate reader.

[0150] For biotinylation of the antibody, Biotin Labeling Kit (CAT# 1 418 165) of Roche was used. A spreadsheet
20 software GlaphPad PRISM (GlaphPad software Inc. ver. 3.0) was used to calculate the concentration of the soluble form of GPC3 in a sample. Fig.5 shows the principle of the sandwich ELISA in this Example.

[0151] Using the purified soluble form of GPC3, a standard curve was prepared. Consequently, a system with a detection limit of several nanograms/mL could be constructed. Fig.6 shows a standard curve for the GPC3 sandwich
25 ELISA using M6B1 and M18D4. Using the system, an attempt was made to detect the secreted form of GPC3 in the culture supernatant of HepG2 and the serum of a mouse transplanted with human hepatoma HepG2. The secreted form of GPC3 was detected in the culture supernatant of HepG2 and the serum of the mouse transplanted with human hepatoma HepG2, while the secreted form of GPC3 was below the detection limit in the control culture medium and the control mouse serum. On a concentration basis of the purified soluble form of GPC3, the soluble form of GPC3
30 was at 1.2 µg/mL in the culture supernatant of HepG2 and at 23 to 90 ng/mL in the serum of the mouse (Table 1).

Table 1

Assay of the secreted form of GPC3 in the plasma of a mouse transplanted with HepG2 (ng/mL)

	Tumor volume (mm ³)	M6B01(N)-M 1BD4(N)	M19B11(N)- M18D4(N)	M6B1(N)- BioM3C11(C)	M13B3(C)-Bi oM18D4(N)	M13B3(C)-Bi oM3B8(C)
Culture supernatant of HepG2		1190	1736	224	234	<1
HepG2-transplanted SCID mouse #1	2022	65.4	76.9	<10	<10	<10
HepG2-transplanted SCID mouse #2	1706	71.7	94.8	<10	<10	<10
HepG2-transplanted SCID mouse #3	2257	90.3	113.9	<10	<10	<10
HepG2-transplanted SCID mouse #4	2081	87.3	107.3	<10	15.0	<10
HepG2-transplanted nude mouse #1	1994	58.7	53.6	19.7	35.5	102.2
HepG2-transplanted nude mouse #2	190 & 549	22.9	33.6	<10	11.5	40.6
Normal SCID mouse #1	0	<10	<10	<10	<10	<10
Normal SCID mouse #2	0	<10	<10	<10	<10	<10
Normal SCID mouse #3	0	<10	<10	<10	<10	<10

Structure of secreted form of GPC3

[0152] It was examined whether or not the blood-secreted GPC3 has the structure of the N-terminal fragment as preliminarily assumed. In case that the secreted form of GPC3 was the N-terminal fragment, it is considered that the secreted form of GPC3 will not be detected by sandwich ELISA with a combination of an antibody recognizing the N terminus and an antibody recognizing the C terminus. Using three types of each antibody recognizing the N-terminal fragment and each antibody recognizing the C-terminal fragment, sandwich ELISA systems with various combinations were constructed. Fig.7 shows the structure of the secreted form of GPC3 and Fig.8 shows combinations of the antibodies. Fig.9 shows a standard curve of the sandwich ELISA. Table 1 shows the assay results. As shown in Table 1, the secreted form of GPC3 was detected at higher values in the culture supernatant of HepG2 and the serum of a mouse transplanted with human hepatoma HepG2 with combinations of antibodies recognizing the N-terminal fragment, while it was detected below the detection limit in many samples from the mice with the systems containing antibodies recognizing the C-terminal fragment. Thus, it was anticipated that the secreted form of GPC3 dominantly comprises the N-terminal fragment. Accordingly, it was suggested that the blood-secreted GPC3 was possibly detected at a high sensitivity by using an antibody against the amino acid sequence comprising the amino acid residue 1 to the amino acid residue 374 of GPC3.

Example 4

Preparation of anti-GPC3 mouse-human chimera antibody

[0153] Using total RNA extracted from a hybridoma producing an antibody capable of binding to human GPC3 (human GPC3-antibody recognizing C-terminus: M3C11, M1E07; human GPC3-antibody recognizing N terminus: M19B11, M18D04, M5B09, M10D02), the cDNA of variable region of the antibody was amplified by RT-PCR. The total RNA was extracted from the hybridoma of 1×10^7 cells, using RNeasy Plant Mini Kits (manufactured by QIAGEN). Using 1 μ g of the total RNA and also using SMART RACE cDNA Amplification Kit (manufactured by CLONTECH), a synthetic oligonucleotide MHC-IgG1 (SEQ ID NO:7) complementary to the mouse IgG1 constant region sequence or a synthetic oligonucleotide kappa (SEQ ID NO:8) complementary to the nucleotide sequence of the mouse κ chain constant region, a 5'-terminal fragment of the gene was amplified. The reverse-transcription was done at 42 °C for one hour and 30 minutes. 50 μ l of the PCR solution contained 5 μ l of $10 \times$ Advantage 2 PCR Buffer, 5 μ l of $10 \times$ Universal Primer A Mix, 0.2 mM dNTPs (dATP, dGTP, dCTP, dTTP), 1 μ l of Advantage 2 Polymerase Mix (all manufactured by CLONTECH), 2.5 μ l of the reverse-transcription product, and 10 pmole of the synthetic oligonucleotide MHC-IgG1 or kappa. After the initial temperature at 94 °C for 30 seconds, a cycle of 94 °C for 5 seconds and 72 °C for 3 minutes was repeated five times; a cycle of 94 °C for 5 seconds, 70 °C for 10 seconds and 72 °C for 3 minutes was repeated five times; and a cycle of 94 °C for 5 seconds, 68 °C for 10 seconds and 72 °C for 3 minutes was repeated 25 times. Finally, the reaction product was heated at 72 °C for 7 minutes. After the individual PCR products were purified from agarose gel using QIAquick Gel Extraction Kit (manufactured by QIAGEN), the products were cloned in pGEM-T Easy vector (manufactured by Promega), and the nucleotide sequence was determined.

[0154] Then, the sequences of the variable regions of the H chain and L chain were linked to the constant regions of the human H chain and L chain. PCR was done using a synthetic oligonucleotide complementary to the 5'-terminal nucleotide sequence of the H chain variable region of each antibody and having the Kozak's sequence and a synthetic oligonucleotide complementary to the 3'-terminal nucleotide sequence and having an NheI site. The resulting PCR products were cloned in a pB-CH vector with the human IgG1 constant region inserted in pBluescript KS+ vector (manufactured by TOYOBO). The mouse H chain variable region and the human H chain (γ 1 chain) constant region are linked together via the NheI site. The prepared H chain gene fragment was cloned in an expression vector pCXND3. The scheme of the construction of the vector pCXND3 is described below. So as to divide the gene encoding the antibody H chain and the vector sequence from DHFR- Δ E-rvH-PM1-f (see WO 92/19759), the vector was digested at the restriction enzyme EcoRI/SmaI sites to recover only the vector sequence. Subsequently, the vector sequence was cloned in EcoRI-NotI-BamHI adaptor (manufactured by Takara Shuzo Co., Ltd.). This vector was designated as pCHO1. A region from pCHO1 expressing the DHFR gene was cloned in pCXN at the restriction enzyme HindIII site (Niwa et al., Gene 1991: 108: 193-200). The resulting vector was designated as pCXND3. The nucleotide sequences of the H chains of the anti-GPC3 mouse-human chimera antibodies (M3C11, M1E07, M19B11, M18D04) contained in each plasmid are shown as SEQ ID NOS: 9, 11, 13 and 15, respectively. The amino acid sequences thereof are shown as SEQ ID NOS: 10, 12, 14, and 16, respectively. Additionally, PCR was done using a synthetic oligonucleotide complementary to the 5'-terminal nucleotide sequence of the L chain variable region of each antibody and having the Kozak's sequence and a synthetic oligonucleotide complementary to the 3'-terminal nucleotide sequence and having a BsiWI site. The resulting PCR products were cloned in a pB-CL vector, where the human kappa chain constant region was preliminarily inserted in pBluescript KS+ vector (manufactured by TOYOBO). The human L chain variable region and

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the constant region were linked together via the BsiWI site. The prepared L chain gene fragment was cloned in an expression vector pUCAG. The vector pUCAG is a vector prepared by digesting pCXN (Niwa et al., Gene 1991: 108: 193-200) with restriction enzyme BamHI to obtain a 2.6-kbp fragment, which is then cloned into the restriction enzyme BamHI site of pUC19 vector (manufactured by TOYOBO). The nucleotide sequences of the L chains of the anti-GPC3 mouse-human chimera antibodies (M3C11, M1E07, M19B11, M18D04) contained in each plasmid are shown as SEQ ID NOS: 17, 19, 21 and 23, respectively. The amino acid sequences thereof are shown as SEQ ID NOS: 18, 20, 22 and 24, respectively.

[0155] So as to prepare an expression vector of the anti-GPC3 mouse-human chimera antibody, a gene fragment obtained by digesting the pUCAG vector having the L chain gene fragment inserted therein with restriction enzyme HindIII (manufactured by Takara Shuzo Co., Ltd.) was cloned into the restriction enzyme HindIII cleavage site of pCXND3 having the H chain gene inserted therein. The plasmid will express the neomycin-resistant gene, the DHFR gene and the anti-GPC3 mouse-human chimera antibody gene in animal cells.

[0156] A CHO-based cell line for stable expression (DG44 line) was prepared as follows. The gene was introduced by electroporation method using Gene PulserII (manufactured by Bio Rad). 25 µg of each expression vector of the anti-GPC3 mouse-human chimera antibody and 0.75 ml of CHO cells (1×10^7 cells/ml) suspended in PBS were mixed together, and cooled on ice for 10 minutes, which was then transferred into a cuvette and received a pulse at 1.5 kV and 25 µFD. After a recovery time at ambient temperature for 10 minutes, the cells treated by the electroporation were suspended in 40 mL of a CHO-S-SFMII culture medium (manufactured by Invitrogen) containing $1 \times$ HT supplement (manufactured by Invitrogen). A 50-fold dilution was prepared using the same culture medium, and added at 100 µl/well in a 96-well culture plate. After culturing in a CO₂ incubator (5 % CO₂) for 24 hours, Geneticin (manufactured by Invitrogen) was added to 0.5 mg/mL, and continued cultivation for 2 weeks. The IgG in the culture supernatant from the wells of colonies of a Geneticin resistance transformant cell was assayed by the following concentration assay method. A cell line with high productivity was expanded at an enlarged scale. The cell line stably expressing the anti-GPC3 mouse-human chimera antibody was cultured in a large-scale culturing and the culture supernatant was collected.

[0157] The IgG concentration in the culture supernatant was assayed by human IgG sandwich ELISA using Goat Anti-human IgG (manufactured by BIOSORCE) and Goat Anti-human IgG alkaline phosphatase conjugated (manufactured by BIOSORCE) and compared with the commercially available purified human IgG (manufactured by Cappel).

[0158] Each anti-GPC3 mouse-human chimera antibody was purified using Hi Trap Protein G HP (manufactured by Amersham). A culture supernatant of a CHO cell line producing the anti-GPC3 mouse-human chimera antibody was directly applied to a column and eluted with elution buffer (0.1 M glycine-HCl, pH 2.7). Eluate was collected into a tube containing a neutralization buffer (1 M Tris-HCl, pH 9.0) for immediate neutralization. Antibody fractions were pooled and dialyzed against 0.05% Tween 20/PBS overnight and for a whole day to replace the buffer. NaN₃ was added to the purified antibody to 0.02 % and stored at 4 °C.

Example 5

Preparation of a CHO cell line stably expressing the full length GPC3

[0159] Human GPC3 cDNA was obtained by digesting pGEM-T Easy vector with the full-length human GPC3 cDNA cloned therein with restriction enzyme EcoRI (manufactured by Takara Shuzo Co., Ltd.) and cloned in an expression vector pCOS2. The scheme of the construction of the vector pCOS2 is described below. So as to divide the gene of the antibody H chain of DHFR-ΔE-rvH-PM1-f (see WO 92/19759) from the vector, the vector was digested at the restriction enzyme EcoRI/SmaI sites, to recover only the vector sequence. Subsequently, the vector sequence was cloned in EcoRI-NotI-BamHI adaptor (manufactured by Takara Shuzo Co., Ltd.). This vector was designated as pCHO1. A region from pCHO1 expressing the DHFR gene was removed, into which the sequence of the neomycin resistant gene in HEF-VH-gy1 (Sato et al., Mol. Immunol. 1994: 31: 371-381) was inserted. The vector was designated as pCOS2.

[0160] A cell line stably expressing the full-length human GPC3 was prepared as follows. 10 µl of the full-length human GPC3 gene-expressing vector and 60 µl of SuperFect (manufactured by QIAGEN) were mixed together, to form a complex, which was then added to a CHO cell line DXB11 to introduce the gene. After culturing in a CO₂ incubator (5 % CO₂) for 24 hours, αMEM (manufactured by GIBCO BRL) containing Geneticin (manufactured by Invitrogen) to a final concentration of 0.5 mg/mL and 10 % FBS (manufactured by GIBCO BRL) was used to start selection. The resulting Geneticin-resistant colonies were collected and cell cloning was done by limited dilution method. Individual cell clones were solubilized to confirm the expression of the full-length human GPC3 by western blotting using the anti-GPC3 antibody. A cell strain stably expressing human GPC3 was obtained.

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Example 6

ADCC assay using PBMC derived from human peripheral blood

5 (1) Preparation of human PBMC

[0161] Peripheral blood was collected from normal subjects with heparinized syringes, and diluted to 2 fold with PBS (-), and overlaid on Ficoll-Paque™ PLUS (Amersham Pharmacia Biotech AB). This was centrifuged ($500 \times g$, 30 minutes, 20°C), and collected the intermediate layer as a mononuclear cell fraction. After rinsing three times, the
10 resulting fraction was suspended in 10 % FBS/RPMI to prepare a human PBMC solution.

(2) Preparation of target cell

[0162] HepG2 cell cultured in 10 % FBS/RPMI 1640 culture medium was detached from the dish using trypsin-EDTA (Invitrogen Corp), divided in each well at 1×10^4 cells/well in a U-bottom 96-well plate (Falcon), and cultured for 2 days. After culturing, 5.55 MBq of chromium-51 was added and the cells were incubated in a 5 % CO_2 gas incubator at 37°C for one hour. The resulting cells were rinsed once with the culture medium, to which 50 μl of 10 % FBS/RPMI 1640 culture medium was added to prepare a target cell.

20 (3) Chromium release test (ADCC activity)

[0163] 50 μl of an antibody solution prepared to each concentration was added to the target cell on ice for 15 minutes. Subsequently, 100 μl of a human PBMC solution was added (5×10^5 cells/well), and incubated in a 5 % CO_2 gas incubator at 37°C for 4 hours. After incubation, the plate was centrifuged and the radioactivity in 100 μl of the culture supernatant was counted with a gamma counter. The specific chromium release ratio was determined by the following formula:

$$\text{Specific chromium release ratio (\%)} = (A - C) \times 100 / (B - C)$$

[0164] "A" represents the mean radioactivity value (cpm) in each well; "B" represents the mean radioactivity value (cpm) in a well where 100 μl of aqueous 2 % NP-40 solution (Nonidet P-40, Code No. 252-23, Nakarai Tesque) and 50 μl of 10 % FBS/RPMI culture medium were added to the target cell; and "C" represents the mean radioactivity value (cpm) in a well where 150 μl of 10 % FBS/RPMI culture medium was added to the target cell. The test was done in triplicate to calculate the mean of the ADCC activity (%) and the standard error.

[0165] The results are shown in Fig.10. Among the six types of anti-GPC3 chimera antibodies, the antibodies ch. M3C11 and ch.M1E07 recognizing the C terminus exerted the ADCC activity, while the antibodies ch. M19B11, ch. M18D04, ch. M5E09 and ch. M10D02 recognizing the N terminus hardly exerted the ADCC activity. The above results indicate that the ADCC activities of the chimera antibodies depend on the recognition sites of the antibodies. Further,
40 it was expected that the antibodies recognizing the C terminus of GPC3 were possibly useful in clinical applications since the antibodies recognizing the C terminal sides from the cleavage sites exerted the ADCC activity.

Example 7

45 Assay of compliment-dependent cytotoxic activity (CDC activity)

(1) Preparation of human albumin veronal buffer (HAVB)

[0166] 12.75 g of NaCl (superior grade; Wako Pure Chemical Industries, Ltd.), 0.5625 g of Na-barbital (superior grade; Wako Pure Chemical Industries, Ltd.), and 0.8625 g of barbital (superior grade; Wako Pure Chemical Industries, Ltd.) were dissolved in Milli Q water to 200 mL, and autoclaved (121°C , 20 minutes). 100 mL of autoclaved warm Milli Q water was added. Then, it was confirmed that the resulting mixture was at pH 7.43 (pH 7.5 recommended). This was defined as 5 \times Veronal Buffer. 0.2205 g of $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$ (superior grade; Wako Pure Chemical Industries, Ltd.) was dissolved in 50 mL of Milli Q water to 0.03 mol/L. The resulting solution was defined as CaCl_2 solution. 1.0165 g of $\text{MgCl}_2 \cdot 6\text{H}_2\text{O}$ (superior grade; Wako Pure Chemical Industries, Ltd.) was dissolved in 50 mL of Milli Q water to 0.1 mol/L. The resulting solution was defined as MgCl_2 solution. 100 mL of 5 \times Veronal Buffer, 4 mL of human serum albumin (Buminat^R 25 %, 250 mg/mL of human serum albumin concentration, Baxter), 2.5 mL of the CaCl_2 solution, 2.5 mL of the MgCl_2 solution, 0.1 g of KCl (superior grade; Wako Pure Chemical Industries, Ltd.), and 0.5 g of glucose

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(D (+)-glucose, anhydrous glucose, superior grade; Wako Pure Chemical Industries, Ltd.) were dissolved in Milli Q water to 500 mL. This was defined as HAVB. After filtration and sterilization, the resulting solution was stored at a set temperature of 5 °C.

5 (2) Preparation of target cell

[0167] CHO cell expressing GPC3 on the cell membrane as prepared in Example 4 was cultured in alpha-MEM nucleic acid (+) culture medium (GIBCO) supplemented with 10 % FBS and 0.5 mg/mL Geneticin (GIBCO), detached from the dish using a cell dissociation buffer (Invitrogen Corp), and divided at 1×10^4 cells/well in each well of a 96-well flat bottom plate (Falcon), for culturing for 3 days. After culturing, 5.55 MBq of chromium-51 was added, and incubated in a 5 % CO₂ gas incubator at 37 °C for one hour. The resulting cell was rinsed twice with HAVE, to which 50 µl of HAVE was added to prepare a target cell.

15 (3) Chromium release test (CDC activity)

[0168] Each chimera antibody was diluted with HAVE to prepare an antibody solution of 40 µg/mL. The antibody solution was added in a 50 µl-portion to the target cell, which was then left on ice for 15 minutes. Subsequently, baby rabbit complement (Cedarlane) diluted with HAVB was added in 100 µl portions to each well to a final concentration of 30 % (final antibody concentration of 10 µg/mL), and incubated in a 5 % CO₂ gas incubator at 37 °C for 90 minutes. After centrifugation of the plate, a 100-µl portion of the supernatant was recovered from each well, and the radioactivity was measured with a gamma counter. The specific chromium release ratio was determined by the following formula:

$$\text{Specific chromium release ratio (\%)} = (A-C) \times 100 / (B-C)$$

[0169] "A" represents the mean radioactivity value (cpm) in each well; "B" represents the mean radioactivity value (cpm) in a well where 100 µl of aqueous 2 % NP-40 solution (Nonidet P-40, Code No. 252-23, Nakarai Tesque) and 50 µl of HAVB were added to the target cell; and "C" represents the mean radioactivity value (cpm) in a well where 150 µl of HAVE was added to the target cell. The test was done in triplicate to calculate the mean of the CDC activity (%) and the standard error.

[0170] The results are shown in Fig.11. Among the six types of the anti-GPC3 chimera antibodies, the antibodies ch.M3C11 and M1E07 recognizing the C terminus exerted the CDC activity, while the antibodies ch. M19B11, ch. M18D04, ch. M5E09 and ch. M10D02 recognizing the N terminus exerted low CDC activities. The above results indicate that the CDC activities of the chimera antibodies depend on the recognition sites of the antibodies. Further, it was expected that the antibodies recognizing the C terminus of GPC3 were possibly useful in clinical applications since the antibodies recognizing the C terminal sides from the cleavage sites exerted the CDC activity.

Industrial Applicability

[0171] As shown in the Examples, it was suggested such that a portion of GPC3 highly expressed in hepatoma cells may exist as a secreted form in blood. Because the gene expression of GPC3 is observed at an earlier stage than that of AFP, a hepatoma marker, GPC3 detection is expected to be useful for cancer diagnosis. It is observed that GPC3 is expressed in cancer cell lines other than hepatoma cell lines, such as lung cancer, colon cancer, breast cancer, prostate cancer, pancreatic cancer and lymphoma. Accordingly, GPC3 is possibly applicable to the diagnosis of cancers other than hepatoma.

[0172] Additionally, it is also suggested that a secreted form of GPC3 in blood predominantly comprises the N-terminal fragment of about 40 kDa, which is observed in the soluble form of the GPC3 core protein. This indicates that antibodies recognizing the N-terminal fragment are useful as the antibody for use in such diagnosis. In addition, if antibodies recognizing the C-terminal fragment with the ADCC activity and/or the CDC activity are used for treating hepatoma, the antibodies can efficiently reach hepatoma cell without being trapped by the secreted form of GPC3 present in blood. Thus, such antibodies are useful as agents for disrupting cancer cells and as anti-cancer agents.

[0173] The contents of all the publications listed in this specification are entirely included in the specification. Additionally, a person skilled in the art will readily understand that various modifications and variations of the invention are possible without departure from the technical scope and inventive range described in the attached claims. It is intended that the invention also encompasses such modifications and variations.

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SEQUENCE LISTING

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 55 60 65

aag ggc cca aca tgc tgc tca aga aag atg gaa gaa aaa tac caa cta 357
 30 Lys Gly Pro Thr Cys Cys Ser Arg Lys Met Glu Glu Lys Tyr Gln Leu
 35 70 75 80

aca gca cga ttg aac atg gaa cag ctg ctt cag tct gca agt atg gag 405
 40 Thr Ala Arg Leu Asn Met Glu Gln Leu Leu Gln Ser Ala Ser Met Glu
 85 90 95

ctc aag ttc tta att att cag aat gct gcg gtt ttc caa gag gcc ttt 453
 45 Leu Lys Phe Leu Ile Ile Gln Asn Ala Ala Val Phe Gln Glu Ala Phe
 100 105 110 115

gaa att gtt gtt cgc cat gcc aag aac tac acc aat gcc atg ttc aag 501
 55

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Glu Ile Val Val Arg His Ala Lys Asn Tyr Thr Asn Ala Met Phe Lys
120 125 130

5
aac aac tac cca agc ctg act cca caa gct ttt gag ttt gtg ggt gaa 549
Asn Asn Tyr Pro Ser Leu Thr Pro Gln Ala Phe Glu Phe Val Gly Glu
10 135 140 145

15
ttt ttc aca gat gtg tct ctc tac atc ttg ggt tct gac atc aat gta 597
Phe Phe Thr Asp Val Ser Leu Tyr Ile Leu Gly Ser Asp Ile Asn Val
150 155 160

20
gat gac atg gtc aat gaa ttg ttt gac agc ctg ttt cca gtc atc tat 645
Asp Asp Met Val Asn Glu Leu Phe Asp Ser Leu Phe Pro Val Ile Tyr
25 165 170 175

30
acc cag cta atg aac cca ggc ctg cct gat tca gcc ttg gac atc aat 693
Thr Gln Leu Met Asn Pro Gly Leu Pro Asp Ser Ala Leu Asp Ile Asn
180 185 190 195

35
gag tgc ctc cga gga gca aga cgt gac ctg aaa gta ttt ggg aat ttc 741
Glu Cys Leu Arg Gly Ala Arg Arg Asp Leu Lys Val Phe Gly Asn Phe
40 200 205 210

45
ccc aag ctt att atg acc cag gtt tcc aag tca ctg caa gtc act agg 789
Pro Lys Leu Ile Met Thr Gln Val Ser Lys Ser Leu Gln Val Thr Arg
215 220 225

50
atc ttc ctt cag gct ctg aat ctt gga att gaa gtg atc aac aca act 837
Ile Phe Leu Gln Ala Leu Asn Leu Gly Ile Glu Val Ile Asn Thr Thr
55

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	230	235	240	
5	gat cac ctg aag ttc agt aag gac tgt ggc cga atg ctc acc aga atg 885			
	Asp His Leu Lys Phe Ser Lys Asp Cys Gly Arg Met Leu Thr Arg Met			
	245	250	255	
10				
	tgg tac tgc tct tac tgc cag gga ctg atg atg gtt aaa ccc tgt ggc 933			
15	Trp Tyr Cys Ser Tyr Cys Gln Gly Leu Met Met Val Lys Pro Cys Gly			
	260	265	270	275
20				
	ggc tac tgc aat gtg gtc atg caa ggc tgt atg gca ggt gtg gtg gag 981			
	Gly Tyr Cys Asn Val Val Met Gln Gly Cys Met Ala Gly Val Val Glu			
	280	285	290	
25				
	att gac aag tac tgg aga gaa tac att ctg tcc ctt gaa gaa ctt gtg 1029			
	Ile Asp Lys Tyr Trp Arg Glu Tyr Ile Leu Ser Leu Glu Glu Leu Val			
30	295	300	305	
35				
	aat ggc atg tac aga atc tat gac atg gag aac gta ctg ctt ggt ctc 1077			
	Asn Gly Met Tyr Arg Ile Tyr Asp Met Glu Asn Val Leu Leu Gly Leu			
	310	315	320	
40				
	ttt tca aca atc cat gat tct atc cag tat gtc cag aag aat gca gga 1125			
	Phe Ser Thr Ile His Asp Ser Ile Gln Tyr Val Gln Lys Asn Ala Gly			
45	325	330	335	
50				
	aag ctg acc acc act att ggc aag tta tgt gcc cat tct caa caa cgc 1173			
	Lys Leu Thr Thr Thr Ile Gly Lys Leu Cys Ala His Ser Gln Gln Arg			
	340	345	350	355
55				

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5 caa tat aga tct gct tat tat cct gaa gat ctc ttt att gac aag aaa 1221
 Gln Tyr Arg Ser Ala Tyr Tyr Pro Glu Asp Leu Phe Ile Asp Lys Lys
 360 365 370

10 gta tta aaa gtt gct cat gta gaa cat gaa gaa acc tta tcc agc cga 1269
 Val Leu Lys Val Ala His Val Glu His Glu Glu Thr Leu Ser Ser Arg
 375 380 385

15 aga agg gaa cta att cag aag ttg aag tct ttc atc agc ttc tat agt 1317
 Arg Arg Glu Leu Ile Gln Lys Leu Lys Ser Phe Ile Ser Phe Tyr Ser
 390 395 400

20 gct ttg cct ggc tac atc tgc agc cat agc cct gtg gcg gaa aac gac 1365
 Ala Leu Pro Gly Tyr Ile Cys Ser His Ser Pro Val Ala Glu Asn Asp
 405 410 415

25 acc ctt tgc tgg aat gga caa gaa ctc gtg gag aga tac agc caa aag 1413
 Thr Leu Cys Trp Asn Gly Gln Glu Leu Val Glu Arg Tyr Ser Gln Lys
 420 425 430 435

30 gca gca agg aat gga atg aaa aac cag ttc aat ctc cat gag ctg aaa 1461
 Ala Ala Arg Asn Gly Met Lys Asn Gln Phe Asn Leu His Glu Leu Lys
 440 445 450

35 atg aag ggc cct gag cca gtg gtc agt caa att att gac aaa ctg aag 1509
 Met Lys Gly Pro Glu Pro Val Val Ser Gln Ile Ile Asp Lys Leu Lys
 455 460 465

40 455 460 465

45 455 460 465

50 455 460 465

55 455 460 465

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5 cac att aac cag ctc ctg aga acc atg tct atg ccc aaa ggt aga gtt 1557
 His Ile Asn Gln Leu Leu Arg Thr Met Ser Met Pro Lys Gly Arg Val
 470 475 480

10 ctg gat aaa aac ctg gat gag gaa ggg ttt gaa agt gga gac tgc ggt 1605
 Leu Asp Lys Asn Leu Asp Glu Glu Gly Phe Glu Ser Gly Asp Cys Gly
 485 490 495

15 gat gat gaa gat gag tgc att gga ggc tct ggt gat gga atg ata aaa 1653
 Asp Asp Glu Asp Glu Cys Ile Gly Gly Ser Gly Asp Gly Met Ile Lys
 20 500 505 510 515

25 gtg aag aat cag ctc cgc ttc ctt gca gaa ctg gcc tat gat ctg gat 1701
 Val Lys Asn Gln Leu Arg Phe Leu Ala Glu Leu Ala Tyr Asp Leu Asp
 520 525 530

30 gtg gat gat gcg cct gga aac agt cag cag gca act ccg aag gac aac 1749
 Val Asp Asp Ala Pro Gly Asn Ser Gln Gln Ala Thr Pro Lys Asp Asn
 35 535 540 545

40 gag ata agc acc ttt cac aac ctc ggg aac gtt cat tcc ccg ctg aag 1797
 Glu Ile Ser Thr Phe His Asn Leu Gly Asn Val His Ser Pro Leu Lys
 550 555 560

45 ctt ctc acc agc atg gcc atc tcg gtg gtg tgc ttc ttc ttc ctg gtg 1845
 Leu Leu Thr Ser Met Ala Ile Ser Val Val Cys Phe Phe Phe Leu Val
 50 565 570 575

55 cac tga ctgcctgggtg cccagcacat gtgctgccct acagaccct gtggtcttcc 1901

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His

580

5

tcgataaagg gaaccacttt cttatttttt tctatttttt tttttttggt atcctgtata 1961

10

cctcctccag ccatgaagta gaggactaac catgtgttat gttttcgaaa atcaaatggt 2021

15

atcitttigga ggaagataca ttttagtggg agcatataga ttgtcctttt gcaaagaaag 2081

20

aaaaaaaaacc atcaagtgtt gccaaattat tctcctatgt ttggctgcta gaacatggtt 2141

accatgtctt tctctctcac tccctccctt tctatcgttc tctctttgca tggatttctt 2201

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tgaaaaaaaa taaattgctc aaataaaaaa aaaaaaaaaa aaaaaaaaaa aaaaaaaaaa 2261

30

aaaaaaaaaa aaaaaaaaaa aaaaaaaaaa aaaaaaaaaa 2300

35

<210> 4

<211> 580

<212> PRT

40

<213> Homo sapiens

<400> 4

45

Met Ala Gly Thr Val Arg Thr Ala Cys Leu Val Val Ala Met Leu Leu

1 5 10 15

50

Ser Leu Asp Phe Pro Gly Gln Ala Gln Pro Pro Pro Pro Pro Asp

20 25 30

Ala Thr Cys His Gln Val Arg Ser Phe Phe Gln Arg Leu Gln Pro Gly

55

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	35	40	45	
5	Leu Lys Trp Val Pro Glu Thr Pro Val Pro Gly Ser Asp Leu Gln Val			
	50	55	60	
	Cys Leu Pro Lys Gly Pro Thr Cys Cys Ser Arg Lys Met Glu Glu Lys			
10	65	70	75	80
	Tyr Gln Leu Thr Ala Arg Leu Asn Met Glu Gln Leu Leu Gln Ser Ala			
	85	90	95	
15	Ser Met Glu Leu Lys Phe Leu Ile Ile Gln Asn Ala Ala Val Phe Gln			
	100	105	110	
	Glu Ala Phe Glu Ile Val Val Arg His Ala Lys Asn Tyr Thr Asn Ala			
20	115	120	125	
	Met Phe Lys Asn Asn Tyr Pro Ser Leu Thr Pro Gln Ala Phe Glu Phe			
25	130	135	140	
	Val Gly Glu Phe Phe Thr Asp Val Ser Leu Tyr Ile Leu Gly Ser Asp			
	145	150	155	160
30	Ile Asn Val Asp Asp Met Val Asn Glu Leu Phe Asp Ser Leu Phe Pro			
	165	170	175	
	Val Ile Tyr Thr Gln Leu Met Asn Pro Gly Leu Pro Asp Ser Ala Leu			
35	180	185	190	
	Asp Ile Asn Glu Cys Leu Arg Gly Ala Arg Arg Asp Leu Lys Val Phe			
	195	200	205	
40	Gly Asn Phe Pro Lys Leu Ile Met Thr Gln Val Ser Lys Ser Leu Gln			
	210	215	220	
	Val Thr Arg Ile Phe Leu Gln Ala Leu Asn Leu Gly Ile Glu Val Ile			
45	225	230	235	240
	Asn Thr Thr Asp His Leu Lys Phe Ser Lys Asp Cys Gly Arg Met Leu			
	245	250	255	
50	Thr Arg Met Trp Tyr Cys Ser Tyr Cys Gln Gly Leu Met Met Val Lys			
	260	265	270	
55				

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Pro Cys Gly Gly Tyr Cys Asn Val Val Met Gln Gly Cys Met Ala Gly
275 280 285

5 Val Val Glu Ile Asp Lys Tyr Trp Arg Glu Tyr Ile Leu Ser Leu Glu
290 295 300

10 Glu Leu Val Asn Gly Met Tyr Arg Ile Tyr Asp Met Glu Asn Val Leu
305 310 315 320

15 Leu Gly Leu Phe Ser Thr Ile His Asp Ser Ile Gln Tyr Val Gln Lys
325 330 335

Asn Ala Gly Lys Leu Thr Thr Thr Ile Gly Lys Leu Cys Ala His Ser
340 345 350

20 Gln Gln Arg Gln Tyr Arg Ser Ala Tyr Tyr Pro Glu Asp Leu Phe Ile
355 360 365

25 Asp Lys Lys Val Leu Lys Val Ala His Val Glu His Glu Glu Thr Leu
370 375 380

Ser Ser Arg Arg Arg Glu Leu Ile Gln Lys Leu Lys Ser Phe Ile Ser
385 390 395 400

30 Phe Tyr Ser Ala Leu Pro Gly Tyr Ile Cys Ser His Ser Pro Val Ala
405 410 415

35 Glu Asn Asp Thr Leu Cys Trp Asn Gly Gln Glu Leu Val Glu Arg Tyr
420 425 430

Ser Gln Lys Ala Ala Arg Asn Gly Met Lys Asn Gln Phe Asn Leu His
435 440 445

40 Glu Leu Lys Met Lys Gly Pro Glu Pro Val Val Ser Gln Ile Ile Asp
450 455 460

45 Lys Leu Lys His Ile Asn Gln Leu Leu Arg Thr Met Ser Met Pro Lys
465 470 475 480

50 Gly Arg Val Leu Asp Lys Asn Leu Asp Glu Glu Gly Phe Glu Ser Gly
485 490 495

Asp Cys Gly Asp Asp Glu Asp Glu Cys Ile Gly Gly Ser Gly Asp Gly

55

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500 505 510
Met Ile Lys Val Lys Asn Gln Leu Arg Phe Leu Ala Glu Leu Ala Tyr
5 515 520 525
Asp Leu Asp Val Asp Asp Ala Pro Gly Asn Ser Gln Gln Ala Thr Pro
530 535 540
10 Lys Asp Asn Glu Ile Ser Thr Phe His Asn Leu Gly Asn Val His Ser
545 550 555 560
15 Pro Leu Lys Leu Leu Thr Ser Met Ala Ile Ser Val Val Cys Phe Phe
565 570 575
Phe Leu Val His
20 580

25

<210> 5

<211> 31

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<212> DNA

<213> Artificial Sequence

35

<220>

<223> Description of Artificial Sequence: Synthetic DNA

40

<400> 5

atagaattcc accatggccg ggaccgtgcg c

31

45

50

<210> 6

<211> 31

<212> DNA

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<213> Artificial Sequence

5 <220>
<223> Description of Artificial Sequence: Synthetic DNA

10 <400> 6
ataggatccc ttcagcgggg aatgaacgtt c 31

15

<210> 7

20 <211> 21
<212> DNA
<213> Artificial Sequence

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<220>
<223> Description of Artificial Sequence: Synthetic DNA

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<400> 7
gggccagtgg atagacagat g 21

35

<210> 8

40 <211> 23
<212> DNA
<213> Artificial Sequence

45

<220>
<223> Description of Artificial Sequence: Synthetic DNA

50

55

5 <400> 8
 gctcactgga tggagggaag atg 23

 10 <210> 9
 <211> 1392
 <212> DNA
 15 <213> Artificial Sequence

 20 <220>
 <221> CDS
 <222> (1).. (1389)
 25
 <220>
 <223> Description of Artificial Sequence: Mouse-human
 30 chimeric antibody (M3C11 H chain)

 <400> 9
 35 atg aac ttc ggg ctc acc ttg att ttc ctt gtc ctt act tta aaa ggt 48
 Met Asn Phe Gly Leu Thr Leu Ile Phe Leu Val Leu Thr Leu Lys Gly
 1 5 10 15
 40
 gtc cag tgt gag gtg caa ctg gtg gag tct ggg gga ggc tta gtg aag 96
 Val Gln Cys Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Lys
 45 20 25 30
 cct gga gga tcc ctg aaa ctc tcc tgt gca gcc tct gga ttc act ttc 144
 Pro Gly Gly Ser Leu Lys Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe
 55 35 40 45

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5 agt cgc tat gcc atg tct tgg gtt cgc cag att cca gag aag ata ctg 192
 Ser Arg Tyr Ala Met Ser Trp Val Arg Gln Ile Pro Glu Lys Ile Leu
 50 55 60

10 gag tgg gtc gca gcc att gat agt agt ggt ggt gac acc tac tat tta 240
 Glu Trp Val Ala Ala Ile Asp Ser Ser Gly Gly Asp Thr Tyr Tyr Leu
 15 65 70 75 80

20 gac act gtg aag gac cga ttc acc atc tcc aga gac aat gcc aat aat 288
 Asp Thr Val Lys Asp Arg Phe Thr Ile Ser Arg Asp Asn Ala Asn Asn
 85 90 95

25 acc ctg cac ctg caa atg cgc agt ctg agg tct gag gac aca gcc ttg 336
 Thr Leu His Leu Gln Met Arg Ser Leu Arg Ser Glu Asp Thr Ala Leu
 30 100 105 110

35 tat tac tgt gta aga cag ggg ggg gct tac tgg ggc caa ggg act ctg 384
 Tyr Tyr Cys Val Arg Gln Gly Gly Ala Tyr Trp Gly Gln Gly Thr Leu
 115 120 125

40 gtc act gtc tct gca gct agc acc aag ggc cca tcg gtc ttc ccc ctg 432
 Val Thr Val Ser Ala Ala Ser Thr Lys Gly Pro Ser Val Phe Pro Leu
 45 130 135 140

50 gca ccc tcc tcc aag agc acc tct ggg ggc aca gcg gcc ctg ggc tgc 480
 Ala Pro Ser Ser Lys Ser Thr Ser Gly Gly Thr Ala Ala Leu Gly Cys
 145 150 155 160

55

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	ctg gtc aag gac tac ttc ccc gaa ccg gtg acg gtg tgg tgg aac tca	528
	Leu Val Lys Asp Tyr Phe Pro Glu Pro Val Thr Val Ser Trp Asn Ser	
5	165 170 175	
	ggc gcc ctg acc agc ggc gtg cac acc ttc ccg gct gtc cta cag tcc	576
10	Gly Ala Leu Thr Ser Gly Val His Thr Phe Pro Ala Val Leu Gln Ser	
	180 185 190	
15	tca gga ctc tac tcc ctc agc agc gtg gtg acc gtg ccc tcc agc agc	624
	Ser Gly Leu Tyr Ser Leu Ser Ser Val Val Thr Val Pro Ser Ser Ser	
20	195 200 205	
	ttg ggc acc cag acc tac atc tgc aac gtg aat cac aag ccc agc aac	672
25	Leu Gly Thr Gln Thr Tyr Ile Cys Asn Val Asn His Lys Pro Ser Asn	
	210 215 220	
30	acc aag gtg gac aag aaa gtt gag ccc aaa tct tgt gac aaa act cac	720
	Thr Lys Val Asp Lys Lys Val Glu Pro Lys Ser Cys Asp Lys Thr His	
35	225 230 235 240	
	aca tgc cca ccg tgc cca gca cct gaa ctc ctg ggg gga ccg tca gtc	768
40	Thr Cys Pro Pro Cys Pro Ala Pro Glu Leu Leu Gly Gly Pro Ser Val	
	245 250 255	
45	ttc ctc ttc ccc cca aaa ccc aag gac acc ctc atg atc tcc cgg acc	816
	Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met Ile Ser Arg Thr	
50	260 265 270	
	cct gag gtc aca tgc glg gtg gtg gac gtg agc cac gaa gac cct gag	864
55		

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Pro Glu Val Thr Cys Val Val Val Asp Val Ser His Glu Asp Pro Glu
275 280 285

5

gtc aag ttc aac tgg tac gtg gac ggc gtg gag gtg cat aat gcc aag 912
Val Lys Phe Asn Trp Tyr Val Asp Gly Val Glu Val His Asn Ala Lys
10 290 295 300

15

aca aag ccg cgg gag gag cag tac aac agc acg tac cgt gtg gtc agc 960
Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser Thr Tyr Arg Val Val Ser
305 310 315 320

20

gtc ctc acc gtc ctg cac cag gac tgg ctg aat ggc aag gag tac aag 1008
Val Leu Thr Val Leu His Gln Asp Trp Leu Asn Gly Lys Glu Tyr Lys
25 325 330 335

30

tgc aag gtc tcc aac aaa gcc ctc cca gcc ccc atc gag aaa acc atc 1056
Cys Lys Val Ser Asn Lys Ala Leu Pro Ala Pro Ile Glu Lys Thr Ile
340 345 350

35

tcc aaa gcc aaa ggg cag ccc cga gaa cca cag gtg tac acc ctg ccc 1104
Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr Thr Leu Pro
40 355 360 365

45

cca tcc cgg gat gag ctg acc aag aac cag gtc agc ctg acc tgc ctg 1152
Pro Ser Arg Asp Glu Leu Thr Lys Asn Gln Val Ser Leu Thr Cys Leu
370 375 380

50

gtc aaa ggc ttc tat ccc agc gac atc gcc gtg gag tgg gag agc aat 1200
Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu Ser Asn
55

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	385	390	395	400	
5	ggg cag ccg gag aac aac tac aag acc acg cct ccc gtg ctg gac tcc				1248
	Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro Val Leu Asp Ser				
10		405	410	415	
	gac ggc tcc ttc ttc ctc tac agc aag ctc acc gtg gac aag agc agg				1296
15	Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr Val Asp Lys Ser Arg				
		420	425	430	
20	tgg cag cag ggg aac gtc ttc tca tgc tcc gtg atg cat gag gct ctg				1344
	Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val Met His Glu Ala Leu				
25		435	440	445	
	cac aac cac tac acg cag aag agc ctc tcc ctg tct ccg ggt aaa tga				1392
30	His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser Pro Gly Lys				
		450	455	460	
35					
	<210> 10				
	<211> 463				
40	<212> PRT				
	<213> Artificial Sequence				
	<223> Description of Artificial Sequence: Mouse-human				
45	chimeric antibody (M3C11 H chain)				
50					
	<400> 10				
	Met Asn Phe Gly Leu Thr Leu Ile Phe Leu Val Leu Thr Leu Lys Gly				
	1	5	10	15	
55					

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5	Val	Gln	Cys	Glu	Val	Gln	Leu	Val	Glu	Ser	Gly	Gly	Gly	Leu	Val	Lys	20	25	30	
10	Pro	Gly	Gly	Ser	Leu	Lys	Leu	Ser	Cys	Ala	Ala	Ser	Gly	Phe	Thr	Phe	35	40	45	
15	Ser	Arg	Tyr	Ala	Met	Ser	Trp	Val	Arg	Gln	Ile	Pro	Glu	Lys	Ile	Leu	50	55	60	
20	Glu	Trp	Val	Ala	Ala	Ile	Asp	Ser	Ser	Gly	Gly	Asp	Thr	Tyr	Tyr	Leu	65	70	75	80
25	Asp	Thr	Val	Lys	Asp	Arg	Phe	Thr	Ile	Ser	Arg	Asp	Asn	Ala	Asn	Asn	85	90	95	
30	Thr	Leu	His	Leu	Gln	Met	Arg	Ser	Leu	Arg	Ser	Glu	Asp	Thr	Ala	Leu	100	105	110	
35	Tyr	Tyr	Cys	Val	Arg	Gln	Gly	Gly	Ala	Tyr	Trp	Gly	Gln	Gly	Thr	Leu	115	120	125	
40	Val	Thr	Val	Ser	Ala	Ala	Ser	Thr	Lys	Gly	Pro	Ser	Val	Phe	Pro	Leu	130	135	140	
45	Ala	Pro	Ser	Ser	Lys	Ser	Thr	Ser	Gly	Gly	Thr	Ala	Ala	Leu	Gly	Cys	145	150	155	160
50	Leu	Val	Lys	Asp	Tyr	Phe	Pro	Glu	Pro	Val	Thr	Val	Ser	Trp	Asn	Ser				

55

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	165	170	175
5	Gly Ala Leu Thr Ser Gly Val His Thr Phe Pro Ala Val Leu Gln Ser		
	180	185	190
10	Ser Gly Leu Tyr Ser Leu Ser Ser Val Val Thr Val Pro Ser Ser Ser		
	195	200	205
15	Leu Gly Thr Gln Thr Tyr Ile Cys Asn Val Asn His Lys Pro Ser Asn		
	210	215	220
20	Thr Lys Val Asp Lys Lys Val Glu Pro Lys Ser Cys Asp Lys Thr His		
	225	230	235
25	Thr Cys Pro Pro Cys Pro Ala Pro Glu Leu Leu Gly Gly Pro Ser Val		
	245	250	255
30	Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met Ile Ser Arg Thr		
	260	265	270
35	Pro Glu Val Thr Cys Val Val Val Asp Val Ser His Glu Asp Pro Glu		
	275	280	285
40	Val Lys Phe Asn Trp Tyr Val Asp Gly Val Glu Val His Asn Ala Lys		
	290	295	300
45	Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser Thr Tyr Arg Val Val Ser		
	305	310	315
50			
	320		
55			

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5	Val Leu Thr Val Leu His Gln Asp Trp Leu Asn Gly Lys Glu Tyr Lys	325	330	335	
10	Cys Lys Val Ser Asn Lys Ala Leu Pro Ala Pro Ile Glu Lys Thr Ile	340	345	350	
15	Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr Thr Leu Pro	355	360	365	
20	Pro Ser Arg Asp Glu Leu Thr Lys Asn Gln Val Ser Leu Thr Cys Leu	370	375	380	
25	Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu Ser Asn	385	390	395	400
30	Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro Val Leu Asp Ser	405	410	415	
35	Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr Val Asp Lys Ser Arg	420	425	430	
40	Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val Met His Glu Ala Leu	435	440	445	
45	His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser Pro Gly Lys	450	455	460	

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5 <210> 11
 <211> 1413
 <212> DNA
 <213> Artificial Sequence
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 <220>
 <221> CDS
 15 <222> (1).. (1410)
 <220>
 20 <223> Description of Artificial Sequence: Mouse-human
 chimeric antibody (M1E07 H chain)
 25
 <400> 11
 atg gga tgg aac tgg atc ttt att tta atc ctg tca gta act aca ggt 48
 30 Met Gly Trp Asn Trp Ile Phe Ile Leu Ile Leu Ser Val Thr Thr Gly
 1 5 10 15
 35 gtc cac tct gag gtc cag ctg cag cag tct gga cct gag ctg gtg aag 96
 Val His Ser Glu Val Gln Leu Gln Gln Ser Gly Pro Glu Leu Val Lys
 20 25 30
 40 cct ggg gct tca gtg aag ata tcc tgc aag gct tct ggt tac tca ttc 144
 Pro Gly Ala Ser Val Lys Ile Ser Cys Lys Ala Ser Gly Tyr Ser Phe
 45 35 40 45
 50 act ggc tac tac atg cac tgg gtg aag caa agt cct gaa aag agc ctt 192
 Thr Gly Tyr Tyr Met His Trp Val Lys Gln Ser Pro Glu Lys Ser Leu
 55 50 55 60

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gag tgg att gga gag att aat cct agc act ggt ggt act acc tac aac 240
 Glu Trp Ile Gly Glu Ile Asn Pro Ser Thr Gly Gly Thr Thr Tyr Asn
 5 65 70 75 80

 cag aag ttc aag gcc aag gcc aca ttg act gta gac aaa tcc tcc agc 288
 Gln Lys Phe Lys Ala Lys Ala Thr Leu Thr Val Asp Lys Ser Ser Ser
 85 90 95
 15
 aca gcc tac atg cag ctc aag agc ctg aca tct gag gac tct gca gtc 336
 Thr Ala Tyr Met Gln Leu Lys Ser Leu Thr Ser Glu Asp Ser Ala Val
 20 100 105 110

 tat tac tgt gca agg agg ggc gga tta act ggg acg agc ttc ttt gct 384
 Tyr Tyr Cys Ala Arg Arg Gly Gly Leu Thr Gly Thr Ser Phe Phe Ala
 25 115 120 125

 tac tgg ggc caa ggg act ctg gtc act gtc tct gca gct agc acc aag 432
 Tyr Trp Gly Gln Gly Thr Leu Val Thr Val Ser Ala Ala Ser Thr Lys
 30 130 135 140

 ggc cca tcg gtc ttc ccc ctg gca ccc tcc tcc aag agc acc tct ggg 480
 Gly Pro Ser Val Phe Pro Leu Ala Pro Ser Ser Lys Ser Thr Ser Gly
 40 145 150 155 160

 ggc aca gcg gcc ctg ggc tgc ctg gtc aag gac tac ttc ccc gaa ccg 528
 Gly Thr Ala Ala Leu Gly Cys Leu Val Lys Asp Tyr Phe Pro Glu Pro
 45 165 170 175
 50
 55

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gtg acg gtg tcg tgg aac tca ggc gcc ctg acc agc ggc gtg cac acc 576
5 Val Thr Val Ser Trp Asn Ser Gly Ala Leu Thr Ser Gly Val His Thr
180 185 190

ttc ccg gct gtc cta cag tcc tca gga ctc tac tcc ctc agc agc gtg 624
10 Phe Pro Ala Val Leu Gln Ser Ser Gly Leu Tyr Ser Leu Ser Ser Val
195 200 205

gtg acc gtg ccc tcc agc agc ttg ggc acc cag acc tac atc tgc aac 672
15 Val Thr Val Pro Ser Ser Ser Leu Gly Thr Gln Thr Tyr Ile Cys Asn
20 210 215 220

gtg aat cac aag ccc agc aac acc aag gtg gac aag aaa gtt gag ccc 720
25 Val Asn His Lys Pro Ser Asn Thr Lys Val Asp Lys Lys Val Glu Pro
225 230 235 240

aaa tct tgt gac aaa act cac aca tgc cca ccg tgc cca gca cct gaa 768
30 Lys Ser Cys Asp Lys Thr His Thr Cys Pro Pro Cys Pro Ala Pro Glu
35 245 250 255

ctc ctg ggg gga ccg tca gtc ttc ctc ttc ccc cca aaa ccc aag gac 816
40 Leu Leu Gly Gly Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp
260 265 270

acc ctc atg atc tcc cgg acc cct gag gtc aca tgc gtg gtg gtg gac 864
45 Thr Leu Met Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp
50 275 280 285

gtg agc cac gaa gac cct gag gtc aag ttc aac tgg tac gtg gac ggc 912
55

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Val Ser His Glu Asp Pro Glu Val Lys Phe Asn Trp Tyr Val Asp Gly
290 295 300

5

gtg gag gtg cat aat gcc aag aca aag ccg cgg gag gag cag tac aac 960
Val Glu Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr Asn
10 305 310 315 320

agc acg tac cgt gtg gtc agc gtc ctc acc gtc ctg cac cag gac tgg 1008
Ser Thr Tyr Arg Val Val Ser Val Leu Thr Val Leu His Gln Asp Trp
15 325 330 335

20

ctg aat ggc aag gag tac aag tgc aag gtc tcc aac aaa gcc ctc cca 1056
Leu Asn Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Ala Leu Pro
25 340 345 350

gcc ccc atc gag aaa acc atc tcc aaa gcc aaa ggg cag ccc cga gaa 1104
Ala Pro Ile Glu Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu
30 355 360 365

35

cca cag gtg tac acc ctg ccc cca tcc cgg gat gag ctg acc aag aac 1152
Pro Gln Val Tyr Thr Leu Pro Pro Ser Arg Asp Glu Leu Thr Lys Asn
40 370 375 380

cag gtc agc ctg acc tgc ctg gtc aaa ggc ttc tat ccc agc gac atc 1200
Gln Val Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile
45 385 390 395 400

50

gcc gtg gag tgg gag agc aat ggg cag ccg gag aac aac tac aag acc 1248
Ala Val Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr
55

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	405	410	415	
5	acg cct ccc gtg ctg gac tcc gac ggc tcc ttc ttc ctc tac agc aag 1296			
	Thr Pro Pro Val Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys			
10	420	425	430	
	ctc acc gtg gac aag agc agg tgg cag cag ggg aac gtc ttc tca tgc 1344			
15	Leu Thr Val Asp Lys Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys			
	435	440	445	
20	tcc gtg atg cat gag gct ctg cac aac cac tac acg cag aag agc ctc 1392			
	Ser Val Met His Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu			
25	450	455	460	
	tcc ctg tct ccg ggt aaa tga 1413			
30	Ser Leu Ser Pro Gly Lys			
	465	470		
35				
	<210> 12			
	<211> 470			
40	<212> PRT			
	<213> Artificial Sequence			
45	<223> Description of Artificial Sequence: Mouse-human			
	chimeric antibody (M1E07 H chain)			
50	<400> 12			
	Met Gly Trp Asn Trp Ile Phe Ile Leu Ile Leu Ser Val Thr Thr Gly			
	1	5	10	15
55				

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5	Val His Ser Glu Val Gln Leu Gln Gln Ser Gly Pro Glu Leu Val Lys	20	25	30
10	Pro Gly Ala Ser Val Lys Ile Ser Cys Lys Ala Ser Gly Tyr Ser Phe	35	40	45
15	Thr Gly Tyr Tyr Met His Trp Val Lys Gln Ser Pro Glu Lys Ser Leu	50	55	60
20	Glu Trp Ile Gly Glu Ile Asn Pro Ser Thr Gly Gly Thr Thr Tyr Asn	65	70	75
25	Gln Lys Phe Lys Ala Lys Ala Thr Leu Thr Val Asp Lys Ser Ser Ser	85	90	95
30	Thr Ala Tyr Met Gln Leu Lys Ser Leu Thr Ser Glu Asp Ser Ala Val	100	105	110
35	Tyr Tyr Cys Ala Arg Arg Gly Gly Leu Thr Gly Thr Ser Phe Phe Ala	115	120	125
40	Tyr Trp Gly Gln Gly Thr Leu Val Thr Val Ser Ala Ala Ser Thr Lys	130	135	140
45	Gly Pro Ser Val Phe Pro Leu Ala Pro Ser Ser Lys Ser Thr Ser Gly	145	150	155
50	Gly Thr Ala Ala Leu Gly Cys Leu Val Lys Asp Tyr Phe Pro Glu Pro			
55				

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	165	170	175
5	Val Thr Val Ser Trp Asn Ser Gly Ala Leu Thr Ser Gly Val His Thr		
	180	185	190
10	Phe Pro Ala Val Leu Gln Ser Ser Gly Leu Tyr Ser Leu Ser Ser Val		
	195	200	205
15	Val Thr Val Pro Ser Ser Ser Leu Gly Thr Gln Thr Tyr Ile Cys Asn		
	210	215	220
20	Val Asn His Lys Pro Ser Asn Thr Lys Val Asp Lys Lys Val Glu Pro		
	225	230	235
25	Lys Ser Cys Asp Lys Thr His Thr Cys Pro Pro Cys Pro Ala Pro Glu		
	245	250	255
30	Leu Leu Gly Gly Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp		
	260	265	270
35	Thr Leu Met Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp		
	275	280	285
40	Val Ser His Glu Asp Pro Glu Val Lys Phe Asn Trp Tyr Val Asp Gly		
	290	295	300
45	Val Glu Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr Asn		
	305	310	315
50			
	320		
55			

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5	Ser Thr Tyr Arg Val Val Ser Val Leu Thr Val Leu His Gln Asp Trp			
		325	330	335
10	Leu Asn Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Ala Leu Pro			
		340	345	350
15	Ala Pro Ile Glu Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu			
		355	360	365
20	Pro Gln Val Tyr Thr Leu Pro Pro Ser Arg Asp Glu Leu Thr Lys Asn			
		370	375	380
25	Gln Val Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile			
		385	390	395
				400
30	Ala Val Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr			
		405	410	415
35	Thr Pro Pro Val Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys			
		420	425	430
40	Leu Thr Val Asp Lys Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys			
		435	440	445
45	Ser Val Met His Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu			
		450	455	460
50	Ser Leu Ser Pro Gly Lys			
		465	470	
55				

5 <210> 13
 <211> 1416
 <212> DNA
 <213> Artificial Sequence
 10 <220>
 <221> CDS
 15 <222> (1).. (1413)
 <220>
 20 <223> Description of Artificial Sequence: Mouse-human
 chimeric antibody (M19B11 H chain)
 25 <400> 13
 atg aac ttc ggg ctc acc ttg att ttc ctc gtc ctt act tta aaa ggt 48
 Met Asn Phe Gly Leu Thr Leu Ile Phe Leu Val Leu Thr Leu Lys Gly
 30 1 5 10 15
 gtc cag tgt gag gtg cag ctg gtg gag tct ggg gga gac tta gtg aag 96
 Val Gln Cys Glu Val Gln Leu Val Glu Ser Gly Gly Asp Leu Val Lys
 35 20 25 30
 40 cct gga ggg acc ctg aaa ctc tcc tgt gca gcc tct gga tcc act ttc 144
 Pro Gly Gly Thr Leu Lys Leu Ser Cys Ala Ala Ser Gly Ser Thr Phe
 45 35 40 45
 50
 55

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5 agt aac tat gcc atg tct tgg gtt cgc cag act cca gag aag agg ctg 192
 Ser Asn Tyr Ala Met Ser Trp Val Arg Gln Thr Pro Glu Lys Arg Leu
 50 55 60

10 gag tgg gtc gca gcc att gat agt aat gga ggt acc acc tac tat cca 240
 Glu Trp Val Ala Ala Ile Asp Ser Asn Gly Gly Thr Thr Tyr Tyr Pro
 65 70 75 80

15 gac act atg aag gac cga ttc acc att tcc aga gac aat gcc aag aac 288
 Asp Thr Met Lys Asp Arg Phe Thr Ile Ser Arg Asp Asn Ala Lys Asn
 20 85 90 95

25 acc ctg tac ctg caa atg aac agt ctg agg tct gaa gac aca gcc ttt 336
 Thr Leu Tyr Leu Gln Met Asn Ser Leu Arg Ser Glu Asp Thr Ala Phe
 100 105 110

30 tat cac tgt aca aga cat aat gga ggg tat gaa aac tac ggc tgg ttt 384
 Tyr His Cys Thr Arg His Asn Gly Gly Tyr Glu Asn Tyr Gly Trp Phe
 35 115 120 125

40 gct tac tgg ggc caa ggg act ctg gtc act gtc tct gca gct agc acc 432
 Ala Tyr Trp Gly Gln Gly Thr Leu Val Thr Val Ser Ala Ala Ser Thr
 130 135 140

45 aag ggc cca tcg gtc ttc ccc ctg gca ccc tcc tcc aag agc acc tct 480
 Lys Gly Pro Ser Val Phe Pro Leu Ala Pro Ser Ser Lys Ser Thr Ser
 50 145 150 155 160

55 ggg ggc aca gcg gcc ctg ggc tgc ctg gtc aag gac tac ttc ccc gaa 528

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Gly Gly Thr Ala Ala Leu Gly Cys Leu Val Lys Asp Tyr Phe Pro Glu
 165 170 175
 5
 ccg gtg acg gtg tgg aac tca ggc gcc ctg acc agc ggc gtg cac 576
 10 Pro Val Thr Val Ser Trp Asn Ser Gly Ala Leu Thr Ser Gly Val His
 180 185 190
 15 acc ttc ccg gct gtc cta cag tcc tca gga ctc tac tcc ctc agc agc 624
 Thr Phe Pro Ala Val Leu Gln Ser Ser Gly Leu Tyr Ser Leu Ser Ser
 195 200 205
 20
 gtg gtg acc gtg ccc tcc agc agc ttg ggc acc cag acc tac atc tgc 672
 25 Val Val Thr Val Pro Ser Ser Ser Leu Gly Thr Gln Thr Tyr Ile Cys
 210 215 220
 30 aac gtg aat cac aag ccc agc aac acc aag gtg gac aag aaa gtt gag 720
 Asn Val Asn His Lys Pro Ser Asn Thr Lys Val Asp Lys Lys Val Glu
 225 230 235 240
 35
 ccc aaa tct tgt gac aaa act cac aca tgc cca ccg tgc cca gca cct 768
 40 Pro Lys Ser Cys Asp Lys Thr His Thr Cys Pro Pro Cys Pro Ala Pro
 245 250 255
 45 gaa ctc ctg ggg gga ccg tca gtc ttc ctc ttc ccc cca aaa ccc aag 816
 Glu Leu Leu Gly Gly Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys
 260 265 270
 50
 gac acc ctc atg atc tcc cgg acc cct gag gtc aca tgc gtg gtg gtg 864
 55 Asp Thr Leu Met Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val Val

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	275	280	285	
5				
	gac gtg agc cac gaa gac cct gag gtc aag ttc aac tgg tac gtg gac			912
	Asp Val Ser His Glu Asp Pro Glu Val Lys Phe Asn Trp Tyr Val Asp			
10	290	295	300	
15				
	ggc gtg gag gtg cat aat gcc aag aca aag ccg cgg gag gag cag tac			960
	Gly Val Glu Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr			
	305	310	315	320
20				
	aac agc acg tac cgt gtg gtc agc gtc ctc acc gtc ctg cac cag gac			1008
	Asn Ser Thr Tyr Arg Val Val Ser Val Leu Thr Val Leu His Gln Asp			
25	325	330	335	
30				
	tgg ctg aat ggc aag gag tac aag tgc aag gtc tcc aac aaa gcc ctc			1056
	Trp Leu Asn Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Ala Leu			
	340	345	350	
35				
	cca gcc ccc atc gag aaa acc atc tcc aaa gcc aaa ggg cag ccc cga			1104
	Pro Ala Pro Ile Glu Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg			
40	355	360	365	
45				
	gaa cca cag gtg tac acc ctg ccc cca tcc cgg gat gag ctg acc aag			1152
	Glu Pro Gln Val Tyr Thr Leu Pro Pro Ser Arg Asp Glu Leu Thr Lys			
	370	375	380	
50				
	aac cag gtc agc ctg acc tgc ctg gtc aaa ggc ttc tat ccc agc gac			1200
	Asn Gln Val Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp			
	385	390	395	400
55				

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atc gcc gtg gag tgg gag agc aat ggg cag ccg gag aac aac tac aag 1248
Ile Ala Val Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys
5 405 410 415

acc acg cct ccc glg ctg gac tcc gac ggc tcc ttc ttc ctc tac agc 1296
Thr Thr Pro Pro Val Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser
10 420 425 430

aag ctc acc gtg gac aag agc agg tgg cag cag ggg aac gtc ttc tca 1344
Lys Leu Thr Val Asp Lys Ser Arg Trp Gln Gln Gly Asn Val Phe Ser
15 435 440 445

tgc tcc gtg atg cat gag gct ctg cac aac cac tac acg cag aag agc 1392
Cys Ser Val Met His Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser
20 450 455 460

ctc tcc ctg tct ccg ggt aaa tga 1416
Leu Ser Leu Ser Pro Gly Lys
25 465 470

<210> 14
<211> 471
<212> PRT
40
45 <213> Artificial Sequence
<223> Description of Artificial Sequence: Mouse-human
chimeric antibody (M19B11 H chain)

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<400> 14

5	Met Asn Phe Gly Leu Thr Leu Ile Phe Leu Val Leu Thr Leu Lys Gly
	1 5 10 15
10	Val Gln Cys Glu Val Gln Leu Val Glu Ser Gly Gly Asp Leu Val Lys
	20 25 30
15	Pro Gly Gly Thr Leu Lys Leu Ser Cys Ala Ala Ser Gly Ser Thr Phe
	35 40 45
20	Ser Asn Tyr Ala Met Ser Trp Val Arg Gln Thr Pro Glu Lys Arg Leu
	50 55 60
25	Glu Trp Val Ala Ala Ile Asp Ser Asn Gly Gly Thr Thr Tyr Tyr Pro
	65 70 75 80
30	Asp Thr Met Lys Asp Arg Phe Thr Ile Ser Arg Asp Asn Ala Lys Asn
	85 90 95
35	Thr Leu Tyr Leu Gln Met Asn Ser Leu Arg Ser Glu Asp Thr Ala Phe
	100 105 110
40	Tyr His Cys Thr Arg His Asn Gly Gly Tyr Glu Asn Tyr Gly Trp Phe
	115 120 125
45	Ala Tyr Trp Gly Gln Gly Thr Leu Val Thr Val Ser Ala Ala Ser Thr
	130 135 140
50	Lys Gly Pro Ser Val Phe Pro Leu Ala Pro Ser Ser Lys Ser Thr Ser
55	

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	145	150	155	160
5	Gly Gly Thr Ala Ala Leu Gly Cys Leu Val Lys Asp Tyr Phe Pro Glu			
	165	170	175	
10	Pro Val Thr Val Ser Trp Asn Ser Gly Ala Leu Thr Ser Gly Val His			
	180	185	190	
15	Thr Phe Pro Ala Val Leu Gln Ser Ser Gly Leu Tyr Ser Leu Ser Ser			
	195	200	205	
20	Val Val Thr Val Pro Ser Ser Ser Leu Gly Thr Gln Thr Tyr Ile Cys			
	210	215	220	
25	Asn Val Asn His Lys Pro Ser Asn Thr Lys Val Asp Lys Lys Val Glu			
	225	230	235	240
30	Pro Lys Ser Cys Asp Lys Thr His Thr Cys Pro Pro Cys Pro Ala Pro			
	245	250	255	
35	Glu Leu Leu Gly Gly Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys			
	260	265	270	
40	Asp Thr Leu Met Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val Val			
	275	280	285	
45	Asp Val Ser His Glu Asp Pro Glu Val Lys Phe Asn Trp Tyr Val Asp			
	290	295	300	
50				
55				

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5	Gly Val Glu Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr			
	305	310	315	320
10	Asn Ser Thr Tyr Arg Val Val Ser Val Leu Thr Val Leu His Gln Asp			
		325	330	335
15	Trp Leu Asn Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Ala Leu			
		340	345	350
20	Pro Ala Pro Ile Glu Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg			
		355	360	365
25	Glu Pro Gln Val Tyr Thr Leu Pro Pro Ser Arg Asp Glu Leu Thr Lys			
		370	375	380
30	Asn Gln Val Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp			
		385	390	395
35	Ile Ala Val Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys			
		405	410	415
40	Thr Thr Pro Pro Val Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser			
		420	425	430
45	Lys Leu Thr Val Asp Lys Ser Arg Trp Gln Gln Gly Asn Val Phe Ser			
		435	440	445
50	Cys Ser Val Met His Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser			
		450	455	460
55				

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Leu Ser Leu Ser Pro Gly Lys
 465 470

<210> 15
 <211> 1413
 <212> DNA
 <213> Artificial Sequence

<220>
 <221> CDS
 <222> (1).. (1410)

<220>
 <223> Description of Artificial Sequence: Mouse-human
 chimeric antibody (M18D04 H chain)

<400> 15
 atg gaa tct aac tgg ata ctt cct ttt att ctg tgc gta gct tca ggg 48
 Met Glu Ser Asn Trp Ile Leu Pro Phe Ile Leu Ser Val Ala Ser Gly
 1 5 10 15
 gtc tac tca gag gtt cag ctc cag cag tct ggg act gtg ctg gca agg 96
 Val Tyr Ser Glu Val Gln Leu Gln Gln Ser Gly Thr Val Leu Ala Arg
 20 25 30
 cct ggg gct tca gtg aag atg tcc tgc aag gct tct ggc tac acc ttt 144

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Pro Gly Ala Ser Val Lys Met Ser Cys Lys Ala Ser Gly Tyr Thr Phe
5 35 40 45

act ggc tac tgg atg cgc tgg gta aaa cag agg cct gga cag ggt ctg 192
10 Thr Gly Tyr Trp Met Arg Trp Val Lys Gln Arg Pro Gly Gln Gly Leu
 50 55 60

gaa tgg att ggc gct att tat cct gga aat agt gat aca aca tac aac 240
15 Glu Trp Ile Gly Ala Ile Tyr Pro Gly Asn Ser Asp Thr Thr Tyr Asn
 65 70 75 80

cag aag ttc aag ggc aag gcc aaa ctg act gca gtc aca tct gtc agc 288
20 Gln Lys Phe Lys Gly Lys Ala Lys Leu Thr Ala Val Thr Ser Val Ser
 85 90 95

act gcc tac atg gaa ctc agc agc ctg aca aat gag gac tct gcg gtc 336
30 Thr Ala Tyr Met Glu Leu Ser Ser Leu Thr Asn Glu Asp Ser Ala Val
 100 105 110

tat tac tgt tca aga tcg ggg gac cta act ggg ggg ttt gct tac tgg 384
35 Tyr Tyr Cys Ser Arg Ser Gly Asp Leu Thr Gly Gly Phe Ala Tyr Trp
40 115 120 125

ggc caa ggg act ctg gtc act gtc tct aca gcc aaa gct agc acc aag 432
45 Gly Gln Gly Thr Leu Val Thr Val Ser Thr Ala Lys Ala Ser Thr Lys
 130 135 140

ggc cca tcg gtc ttc ccc ctg gca ccc tcc tcc aag agc acc tct ggg 480
50 Gly Pro Ser Val Phe Pro Leu Ala Pro Ser Ser Lys Ser Thr Ser Gly
55

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	145	150	155	160	
5	ggc aca gcg gcc ctg ggc tgc ctg gtc aag gac tac ttc ccc gaa ccg				528
	Gly Thr Ala Ala Leu Gly Cys Leu Val Lys Asp Tyr Phe Pro Glu Pro				
		165	170	175	
10					
	gtg acg gtg tgc tgg aac tca ggc gcc ctg acc agc ggc gtg cac acc				576
15	Val Thr Val Ser Trp Asn Ser Gly Ala Leu Thr Ser Gly Val His Thr				
		180	185	190	
20					
	ttc ccg gct gtc cta cag tcc tca gga ctc tac tcc ctc agc agc gtg				624
	Phe Pro Ala Val Leu Gln Ser Ser Gly Leu Tyr Ser Leu Ser Ser Val				
		195	200	205	
25					
	gtg acc gtg ccc tcc agc agc ttg ggc acc cag acc tac atc tgc aac				672
30	Val Thr Val Pro Ser Ser Ser Leu Gly Thr Gln Thr Tyr Ile Cys Asn				
		210	215	220	
35					
	gtg aat cac aag ccc agc aac acc aag gtg gac aag aaa gtt gag ccc				720
	Val Asn His Lys Pro Ser Asn Thr Lys Val Asp Lys Lys Val Glu Pro				
		225	230	235	240
40					
	aaa tct tgt gac aaa act cac aca tgc cca ccg tgc cca gca cct gaa				768
	Lys Ser Cys Asp Lys Thr His Thr Cys Pro Pro Cys Pro Ala Pro Glu				
45		245	250	255	
50					
	ctc ctg ggg gga ccg tca gtc ttc ctc ttc ccc cca aaa ccc aag gac				816
	Leu Leu Gly Gly Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp				
		260	265	270	
55					

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acc ctc atg atc tcc cgg acc cct gag gtc aca tgc gtg gtg gtg gac 864
 Thr Leu Met Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp
 5 275 280 285

gtg agc cac gaa gac cct gag gtc aag ttc aac tgg tac gtg gac ggc 912
 Val Ser His Glu Asp Pro Glu Val Lys Phe Asn Trp Tyr Val Asp Gly
 10 290 295 300

gtg gag gtg cat aat gcc aag aca aag ccg cgg gag gag cag tac aac 960
 Val Glu Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr Asn
 15 20 305 310 315 320

agc acg tac cgt gtg gtc agc gtc ctc acc gtc ctg cac cag gac tgg 1008
 Ser Thr Tyr Arg Val Val Ser Val Leu Thr Val Leu His Gln Asp Trp
 25 325 330 335

ctg aat ggc aag gag tac aag tgc aag gtc tcc aac aaa gcc ctc cca 1056
 Leu Asn Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Ala Leu Pro
 30 35 340 345 350

gcc ccc atc gag aaa acc atc tcc aaa gcc aaa ggg cag ccc cga gaa 1104
 Ala Pro Ile Glu Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu
 40 355 360 365

cca cag gtg tac acc ctg ccc cca tcc cgg gat gag ctg acc aag aac 1152
 Pro Gln Val Tyr Thr Leu Pro Pro Ser Arg Asp Glu Leu Thr Lys Asn
 45 50 370 375 380

55

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5 cag gtc agc ctg acc tgc ctg gtc aaa ggc ttc tat ccc agc gac atc 1200
Gln Val Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile
385 390 395 400

10 gcc gtg gag tgg gag agc aat ggg cag ccg gag aac aac tac aag acc 1248
Ala Val Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr
405 410 415

15 acg cct ccc gtg ctg gac tcc gac ggc tcc ttc ttc ctc tac agc aag 1296
Thr Pro Pro Val Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys
20 420 425 430

25 ctc acc gtg gac aag agc agg tgg cag cag ggg aac gtc ttc tca tgc 1344
Leu Thr Val Asp Lys Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys
435 440 445

30 tcc gtg atg cat gag gct ctg cac aac cac tac acg cag aag agc ctc 1392
Ser Val Met His Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu
35 450 455 460

40 tcc ctg tct ccg ggt aaa tga 1413
Ser Leu Ser Pro Gly Lys
465 470

45

50 <210> 16
<211> 470
<212> PRT
<213> Artificial Sequence

55

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<223> Description of Artificial Sequence: Mouse-human
chimeric antibody (M18D04 H chain)

<400> 16

Met Glu Ser Asn Trp Ile Leu Pro Phe Ile Leu Ser Val Ala Ser Gly
1 5 10 15

Val Tyr Ser Glu Val Gln Leu Gln Gln Ser Gly Thr Val Leu Ala Arg
20 25 30

Pro Gly Ala Ser Val Lys Met Ser Cys Lys Ala Ser Gly Tyr Thr Phe
35 40 45

Thr Gly Tyr Trp Met Arg Trp Val Lys Gln Arg Pro Gly Gln Gly Leu
50 55 60

Glu Trp Ile Gly Ala Ile Tyr Pro Gly Asn Ser Asp Thr Thr Tyr Asn
65 70 75 80

Gln Lys Phe Lys Gly Lys Ala Lys Leu Thr Ala Val Thr Ser Val Ser
85 90 95

Thr Ala Tyr Met Glu Leu Ser Ser Leu Thr Asn Glu Asp Ser Ala Val
100 105 110

Tyr Tyr Cys Ser Arg Ser Gly Asp Leu Thr Gly Gly Phe Ala Tyr Trp
115 120 125

Gly Gln Gly Thr Leu Val Thr Val Ser Thr Ala Lys Ala Ser Thr Lys

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	130	135	140	
5	Gly Pro Ser Val Phe Pro Leu Ala Pro Ser Ser Lys Ser Thr Ser Gly			
	145	150	155	160
10	Gly Thr Ala Ala Leu Gly Cys Leu Val Lys Asp Tyr Phe Pro Glu Pro			
	165	170	175	
15	Val Thr Val Ser Trp Asn Ser Gly Ala Leu Thr Ser Gly Val His Thr			
	180	185	190	
20	Phe Pro Ala Val Leu Gln Ser Ser Gly Leu Tyr Ser Leu Ser Ser Val			
	195	200	205	
25	Val Thr Val Pro Ser Ser Ser Leu Gly Thr Gln Thr Tyr Ile Cys Asn			
	210	215	220	
30	Val Asn His Lys Pro Ser Asn Thr Lys Val Asp Lys Lys Val Glu Pro			
	225	230	235	240
35	Lys Ser Cys Asp Lys Thr His Thr Cys Pro Pro Cys Pro Ala Pro Glu			
	245	250	255	
40	Leu Leu Gly Gly Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp			
	260	265	270	
45	Thr Leu Met Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp			
	275	280	285	
50				
55				

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5	Val Ser His Glu Asp Pro Glu Val Lys Phe Asn Trp Tyr Val Asp Gly	290	295	300
10	Val Glu Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr Asn	305	310	315 320
15	Ser Thr Tyr Arg Val Val Ser Val Leu Thr Val Leu His Gln Asp Trp	325	330	335
20	Leu Asn Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Ala Leu Pro	340	345	350
25	Ala Pro Ile Glu Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu	355	360	365
30	Pro Gln Val Tyr Thr Leu Pro Pro Ser Arg Asp Glu Leu Thr Lys Asn	370	375	380
35	Gln Val Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile	385	390	395 400
40	Ala Val Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr	405	410	415
45	Thr Pro Pro Val Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys	420	425	430
50	Leu Thr Val Asp Lys Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys	435	440	445
55				

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5 Ser Val Met His Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu
 450 455 460

10 Ser Leu Ser Pro Gly Lys
 465 470

15

20 <210> 17
 <211> 717
 <212> DNA
 <213> Artificial Sequence

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 <220>
 <221> CDS
 30 <222> (1).. (714)

35 <220>
 <223> Description of Artificial Sequence: Mouse-human
 chimeric antibody (M3C11 L chain)

40

 <400> 17
 atg agt cct gcc cag ttc ctg ttt ctg tta gtg ctc tgg att cgg gaa 48
 45 Met Ser Pro Ala Gln Phe Leu Phe Leu Leu Val Leu Trp Ile Arg Glu
 1 5 10 15

50 acc aac ggt gat gtt gtg atg acc cag act cca ctc act ttg tgg gtt 96
 Thr Asn Gly Asp Val Val Met Thr Gln Thr Pro Leu Thr Leu Ser Val

55

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	20	25	30	
5	acc att gga caa cca gcc tcc atc tct tgc aag tca agt cag agc ctc 144			
	Thr Ile Gly Gln Pro Ala Ser Ile Ser Cys Lys Ser Ser Gln Ser Leu			
10	35	40	45	
15	tta gat agt gat gga aag aca tat ttg aat tgg ttg tta cag agg cca 192			
	Leu Asp Ser Asp Gly Lys Thr Tyr Leu Asn Trp Leu Leu Gln Arg Pro			
	50	55	60	
20	ggc cag tct cca aag cgc cta atc tat ctg gtc tct aaa ttg gac tct 240			
	Gly Gln Ser Pro Lys Arg Leu Ile Tyr Leu Val Ser Lys Leu Asp Ser			
25	65	70	75	80
30	gga gcc cct gac agg ttc act ggc agt gga tca ggg aca gat ttc aca 288			
	Gly Ala Pro Asp Arg Phe Thr Gly Ser Gly Ser Gly Thr Asp Phe Thr			
	85	90	95	
35	ctg aaa atc agt aga gtc gag gct gag gat ttg gga att tat tat tgc 336			
	Leu Lys Ile Ser Arg Val Glu Ala Glu Asp Leu Gly Ile Tyr Tyr Cys			
40	100	105	110	
45	tgg caa ggt aca cat ttt ccg ctc acg ttc ggt gct ggg acc aag ctg 384			
	Trp Gln Gly Thr His Phe Pro Leu Thr Phe Gly Ala Gly Thr Lys Leu			
	115	120	125	
50	gag ctg aaa cgt acg gtc gct gca cca tct gtc ttc atc ttc ccg cca 432			
	Glu Leu Lys Arg Thr Val Ala Ala Pro Ser Val Phe Ile Phe Pro Pro			
55	130	135	140	

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5 tct gat gag cag ttg aaa tct gga act gcc tct gtt gtg tgc ctg ctg 480
 Ser Asp Glu Gln Leu Lys Ser Gly Thr Ala Ser Val Val Cys Leu Leu
 145 150 155 160

10 aat aac ttc tat ccc aga gag gcc aaa gta cag tgg aag gtg gat aac 528
 Asn Asn Phe Tyr Pro Arg Glu Ala Lys Val Gln Trp Lys Val Asp Asn
 15 165 170 175

20 gcc ctc caa tgc ggt aac tcc cag gag agt gtc aca gag cag gac agc 576
 Ala Leu Gln Ser Gly Asn Ser Gln Glu Ser Val Thr Glu Gln Asp Ser
 180 185 190

25 aag gac agc acc tac agc ctc agc agc acc ctg acg ctg agc aaa gca 624
 Lys Asp Ser Thr Tyr Ser Leu Ser Ser Thr Leu Thr Leu Ser Lys Ala
 195 200 205

30 gac tac gag aaa cac aaa gtc tac gcc tgc gaa gtc acc cat cag ggc 672
 Asp Tyr Glu Lys His Lys Val Tyr Ala Cys Glu Val Thr His Gln Gly
 35 210 215 220

40 ctg agc tgc ccc gtc aca aag agc ttc aac agg gga gag tgt tga 717
 Leu Ser Ser Pro Val Thr Lys Ser Phe Asn Arg Gly Glu Cys
 225 230 235

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50 <210> 18
 <211> 238
 <212> PRT

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<213> Artificial Sequence

<223> Description of Artificial Sequence: Mouse-human
chimeric antibody (M3C11 L chain)

<400> 18

Met Ser Pro Ala Gln Phe Leu Phe Leu Leu Val Leu Trp Ile Arg Glu

1 5 10 15

Thr Asn Gly Asp Val Val Met Thr Gln Thr Pro Leu Thr Leu Ser Val

20 25 30

Thr Ile Gly Gln Pro Ala Ser Ile Ser Cys Lys Ser Ser Gln Ser Leu

35 40 45

Leu Asp Ser Asp Gly Lys Thr Tyr Leu Asn Trp Leu Leu Gln Arg Pro

50 55 60

Gly Gln Ser Pro Lys Arg Leu Ile Tyr Leu Val Ser Lys Leu Asp Ser

65 70 75 80

Gly Ala Pro Asp Arg Phe Thr Gly Ser Gly Ser Gly Thr Asp Phe Thr

85 90 95

Leu Lys Ile Ser Arg Val Glu Ala Glu Asp Leu Gly Ile Tyr Tyr Cys

100 105 110

Trp Gln Gly Thr His Phe Pro Leu Thr Phe Gly Ala Gly Thr Lys Leu

115 120 125

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5 Glu Leu Lys Arg Thr Val Ala Ala Pro Ser Val Phe Ile Phe Pro Pro
 130 135 140

 10 Ser Asp Glu Gln Leu Lys Ser Gly Thr Ala Ser Val Val Cys Leu Leu
 145 150 155 160

 15 Asn Asn Phe Tyr Pro Arg Glu Ala Lys Val Gln Trp Lys Val Asp Asn
 165 170 175

 20 Ala Leu Gln Ser Gly Asn Ser Gln Glu Ser Val Thr Glu Gln Asp Ser
 180 185 190

 25 Lys Asp Ser Thr Tyr Ser Leu Ser Ser Thr Leu Thr Leu Ser Lys Ala
 195 200 205

 30 Asp Tyr Glu Lys His Lys Val Tyr Ala Cys Glu Val Thr His Gln Gly
 210 215 220

 35 Leu Ser Ser Pro Val Thr Lys Ser Phe Asn Arg Gly Glu Cys
 225 230 235

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 45 <210> 19
 <211> 717
 <212> DNA
 50 <213> Artificial Sequence

 55 <220>

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<221> CDS

<222> (1).. (714)

5

<220>

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<223> Description of Artificial Sequence: Mouse-human
chimeric antibody (M1E07 L chain)

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<400> 19

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Met Ser Pro Val Gln Phe Leu Phe Leu Leu Met Leu Trp Ile Gln Glu

20

1

5

10

15

acc aac ggt gat gtt gtg atg acc cag act cca ctg tct ttg tgc gtt 96

25

Thr Asn Gly Asp Val Val Met Thr Gln Thr Pro Leu Ser Leu Ser Val

20

25

30

30

acc att gga caa cca gcc tct atc tct tgc aag tca agt cag agc ctc 144

Thr Ile Gly Gln Pro Ala Ser Ile Ser Cys Lys Ser Ser Gln Ser Leu

35

35

40

45

tta tat agt aat gga aag aca tat ttg aat tgg tta caa cag agg cct 192

40

Leu Tyr Ser Asn Gly Lys Thr Tyr Leu Asn Trp Leu Gln Gln Arg Pro

50

55

60

45

ggc cag gct cca aag cac cta atg tat cag gtg tcc aaa ctg gac cct 240

Gly Gln Ala Pro Lys His Leu Met Tyr Gln Val Ser Lys Leu Asp Pro

50

65

70

75

80

ggc atc cct gac agg ttc agt ggc agt gga tca gaa aca gat ttt aca 288

55

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5 Gly Ile Pro Asp Arg Phe Ser Gly Ser Gly Ser Glu Thr Asp Phe Thr
85 90 95

ctt aaa atc agc aga gtg gag gct gaa gat ttg gga gtt tat tac tgc 336
10 Leu Lys Ile Ser Arg Val Glu Ala Glu Asp Leu Gly Val Tyr Tyr Cys
100 105 110

15 ttg caa agt aca tat tat ccg ctc acg ttc ggt gct ggg acc aag ctg 384
Leu Gln Ser Thr Tyr Tyr Pro Leu Thr Phe Gly Ala Gly Thr Lys Leu
115 120 125

20 gag ctg aaa cgt acg gtg gct gca cca tct gtc ttc atc ttc ccg cca 432
Glu Leu Lys Arg Thr Val Ala Ala Pro Ser Val Phe Ile Phe Pro Pro
25 130 135 140

30 tct gat gag cag ttg aaa tct gga act gcc tct gtt gtg tgc ctg ctg 480
Ser Asp Glu Gln Leu Lys Ser Gly Thr Ala Ser Val Val Cys Leu Leu
145 150 155 160

35 aat aac ttc tat ccc aga gag gcc aaa gta cag tgg aag gtg gat aac 528
Asn Asn Phe Tyr Pro Arg Glu Ala Lys Val Gln Trp Lys Val Asp Asn
40 165 170 175

45 gcc ctc caa tcg ggt aac tcc cag gag agt gtc aca gag cag gac agc 576
Ala Leu Gln Ser Gly Asn Ser Gln Glu Ser Val Thr Glu Gln Asp Ser
180 185 190

50 aag gac agc acc tac agc ctc agc agc acc ctg acg ctg agc aaa gca 624
Lys Asp Ser Thr Tyr Ser Leu Ser Ser Thr Leu Thr Leu Ser Lys Ala
55

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195 200 205

5 gac tac gag aaa cac aaa gtc tac gcc tgc gaa gtc acc cat cag ggc 672
 Asp Tyr Glu Lys His Lys Val Tyr Ala Cys Glu Val Thr His Gln Gly

10 210 215 220

15 ctg agc tcg ccc gtc aca aag agc ttc aac agg gga gag tgt tga 717
 Leu Ser Ser Pro Val Thr Lys Ser Phe Asn Arg Gly Glu Cys

225 230 235

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<210> 20

<211> 238

25 <212> PRT

<213> Artificial Sequence

30 <223> Description of Artificial Sequence: Mouse-human
 chimeric antibody (M1E07 L chain)

35 <400> 20

Met Ser Pro Val Gln Phe Leu Phe Leu Leu Met Leu Trp Ile Gln Glu

1 5 10 15

40 Thr Asn Gly Asp Val Val Met Thr Gln Thr Pro Leu Ser Leu Ser Val

20 25 30

45 Thr Ile Gly Gln Pro Ala Ser Ile Ser Cys Lys Ser Ser Gln Ser Leu

35 40 45

50 Leu Tyr Ser Asn Gly Lys Thr Tyr Leu Asn Trp Leu Gln Gln Arg Pro

55

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	50	55	60	
5	Gly Gln Ala Pro Lys His Leu Met Tyr Gln Val Ser Lys Leu Asp Pro			
	65	70	75	80
10	Gly Ile Pro Asp Arg Phe Ser Gly Ser Gly Ser Glu Thr Asp Phe Thr			
		85	90	95
15	Leu Lys Ile Ser Arg Val Glu Ala Glu Asp Leu Gly Val Tyr Tyr Cys			
	100	105	110	
20	Leu Gln Ser Thr Tyr Tyr Pro Leu Thr Phe Gly Ala Gly Thr Lys Leu			
	115	120	125	
25	Glu Leu Lys Arg Thr Val Ala Ala Pro Ser Val Phe Ile Phe Pro Pro			
	130	135	140	
30	Ser Asp Glu Gln Leu Lys Ser Gly Thr Ala Ser Val Val Cys Leu Leu			
	145	150	155	160
35	Asn Asn Phe Tyr Pro Arg Glu Ala Lys Val Gln Trp Lys Val Asp Asn			
		165	170	175
40	Ala Leu Gln Ser Gly Asn Ser Gln Glu Ser Val Thr Glu Gln Asp Ser			
	180	185	190	
45	Lys Asp Ser Thr Tyr Ser Leu Ser Ser Thr Leu Thr Leu Ser Lys Ala			
	195	200	205	
50				
55				

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Asp Tyr Glu Lys His Lys Val Tyr Ala Cys Glu Val Thr His Gln Gly

210

215

220

5

Leu Ser Ser Pro Val Thr Lys Ser Phe Asn Arg Gly Glu Cys

225

230

235

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<210> 21

<211> 705

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<212> DNA

<213> Artificial Sequence

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<220>

<221> CDS

<222> (1).. (702)

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<220>

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<223> Description of Artificial Sequence: Mouse-human
chimeric antibody (M19B11 L chain)

40

<400> 21

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Met Arg Pro Ser Ile Gln Phe Leu Gly Leu Leu Leu Phe Trp Leu His

45

1

5

10

15

50

ggt gtt cag tgt gac atc cag atg aca cag tct cca tcc tca ctg tct 96

Gly Val Gln Cys Asp Ile Gln Met Thr Gln Ser Pro Ser Ser Leu Ser

20

25

30

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5 gca tct ctg gga ggc aaa gtc acc atc act tgc aag gca agt cag gac 144
Ala Ser Leu Gly Gly Lys Val Thr Ile Thr Cys Lys Ala Ser Gln Asp
35 40 45

10 att aac aag aat ata gtt tgg tac caa cac aag cct gga aaa ggt cct 192
Ile Asn Lys Asn Ile Val Trp Tyr Gln His Lys Pro Gly Lys Gly Pro
15 50 55 60

20 agg ctg ctc ata tgg tac aca tct aca tta cag cca ggc atc cca tca 240
Arg Leu Leu Ile Trp Tyr Thr Ser Thr Leu Gln Pro Gly Ile Pro Ser
65 70 75 80

25 agg ttc agt gga agt ggg tct ggg aga gat tat tcc ttc agc atc agc 288
Arg Phe Ser Gly Ser Gly Ser Gly Arg Asp Tyr Ser Phe Ser Ile Ser
30 85 90 95

35 aac ctg gag cct gaa gat att gca act tat tac tgt cta cag tat gat 336
Asn Leu Glu Pro Glu Asp Ile Ala Thr Tyr Tyr Cys Leu Gln Tyr Asp
100 105 110

40 aat ctt cca cgg acg ttc ggt gga ggc acc aaa ctg gaa atc aaa cgt 384
Asn Leu Pro Arg Thr Phe Gly Gly Gly Thr Lys Leu Glu Ile Lys Arg
115 120 125

45 acg gtg gct gca cca tct gtc ttc atc ttc ccg cca tct gat gag cag 432
Thr Val Ala Ala Pro Ser Val Phe Ile Phe Pro Pro Ser Asp Glu Gln
50 130 135 140

55

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5 ttg aaa tct gga act gcc tct gtt gtg tgc ctg ctg aat aac ttc tat 480
 Leu Lys Ser Gly Thr Ala Ser Val Val Cys Leu Leu Asn Asn Phe Tyr
 145 150 155 160

10 ccc aga gag gcc aaa gta cag tgg aag gtg gat aac gcc ctc caa tcg 528
 Pro Arg Glu Ala Lys Val Gln Trp Lys Val Asp Asn Ala Leu Gln Ser
 165 170 175

15 ggt aac tcc cag gag agt gtc aca gag cag gac agc aag gac agc acc 576
 Gly Asn Ser Gln Glu Ser Val Thr Glu Gln Asp Ser Lys Asp Ser Thr
 180 185 190

20 tac agc ctc agc agc acc ctg acg ctg agc aaa gca gac tac gag aaa 624
 Tyr Ser Leu Ser Ser Thr Leu Thr Leu Ser Lys Ala Asp Tyr Glu Lys
 195 200 205

25 cac aaa gtc tac gcc tgc gaa gtc acc cat cag ggc ctg agc tcg ccc 672
 His Lys Val Tyr Ala Cys Glu Val Thr His Gln Gly Leu Ser Ser Pro
 210 215 220

30 gtc aca aag agc ttc aac agg gga gag tgt tga 705
 Val Thr Lys Ser Phe Asn Arg Gly Glu Cys
 225 230

35 <210> 22

40 <211> 234

45 <212> PRT

50 <213> Artificial Sequence

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<223> Description of Artificial Sequence: Mouse-human
chimeric antibody (M19B11 L chain)

<400> 22

Met Arg Pro Ser Ile Gln Phe Leu Gly Leu Leu Leu Phe Trp Leu His
1 5 10 15

Gly Val Gln Cys Asp Ile Gln Met Thr Gln Ser Pro Ser Ser Leu Ser
20 25 30

Ala Ser Leu Gly Gly Lys Val Thr Ile Thr Cys Lys Ala Ser Gln Asp
35 40 45

Ile Asn Lys Asn Ile Val Trp Tyr Gln His Lys Pro Gly Lys Gly Pro
50 55 60

Arg Leu Leu Ile Trp Tyr Thr Ser Thr Leu Gln Pro Gly Ile Pro Ser
65 70 75 80

Arg Phe Ser Gly Ser Gly Ser Gly Arg Asp Tyr Ser Phe Ser Ile Ser
85 90 95

Asn Leu Glu Pro Glu Asp Ile Ala Thr Tyr Tyr Cys Leu Gln Tyr Asp
100 105 110

Asn Leu Pro Arg Thr Phe Gly Gly Gly Thr Lys Leu Glu Ile Lys Arg
115 120 125

Thr Val Ala Ala Pro Ser Val Phe Ile Phe Pro Pro Ser Asp Glu Gln

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	130	135	140
5	Leu Lys Ser Gly Thr Ala Ser Val Val Cys Leu Leu Asn Asn Phe Tyr		
	145	150	155 160
10	Pro Arg Glu Ala Lys Val Gln Trp Lys Val Asp Asn Ala Leu Gln Ser		
	165	170	175
15	Gly Asn Ser Gln Glu Ser Val Thr Glu Gln Asp Ser Lys Asp Ser Thr		
	180	185	190
20	Tyr Ser Leu Ser Ser Thr Leu Thr Leu Ser Lys Ala Asp Tyr Glu Lys		
	195	200	205
25	His Lys Val Tyr Ala Cys Glu Val Thr His Gln Gly Leu Ser Ser Pro		
	210	215	220
30	Val Thr Lys Ser Phe Asn Arg Gly Glu Cys		
	225	230	
35			
40			
	<210> 23		
	<211> 720		
45	<212> DNA		
	<213> Artificial Sequence		
50	<220>		
	<221> CDS		
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<222> (1).. (717)

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<220>

<223> Description of Artificial Sequence: Mouse-human

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chimeric antibody (M18D04 L chain)

<400> 23

15

atg agg ttc tct gct cag ctt ctg ggg ctg ctt gtg ctc tgg atc cct 48

Met Arg Phe Ser Ala Gln Leu Leu Gly Leu Leu Val Leu Trp Ile Pro

1 5 10 15

20

gga tcc act gca gat att gtg atg acg cag gct gca ttc tcc aat cca 96

Gly Ser Thr Ala Asp Ile Val Met Thr Gln Ala Ala Phe Ser Asn Pro

25

20 25 30

30

gtc act ctt gga aca tca act tcc atc tcc tgc agg tct agt aag agt 144

Val Thr Leu Gly Thr Ser Thr Ser Ile Ser Cys Arg Ser Ser Lys Ser

35 40 45

35

ctc cta cat agt aat ggc atc act tat ttg tat tgg tat ctg cag aag 192

Leu Leu His Ser Asn Gly Ile Thr Tyr Leu Tyr Trp Tyr Leu Gln Lys

40

50 55 60

45

cca ggc cag tct cct cag ctc ctg att tat cag atg tcc aac ctt gcc 240

Pro Gly Gln Ser Pro Gln Leu Leu Ile Tyr Gln Met Ser Asn Leu Ala

65 70 75 80

50

tca gga gtc cca gac agg ttc agt agc agt ggg tca gga act gat ttc 288

Ser Gly Val Pro Asp Arg Phe Ser Ser Ser Gly Ser Gly Thr Asp Phe

55

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	85	90	95	
5				
	aca ctg aga atc agc aga gtg gag gct gag gat gtg ggt gtt tat tac			336
	Thr Leu Arg Ile Ser Arg Val Glu Ala Glu Asp Val Gly Val Tyr Tyr			
10	100	105	110	
	tgt gct caa aat cta gaa ctt ccg tat acg ttc gga tcg ggg acc aag			384
15	Cys Ala Gln Asn Leu Glu Leu Pro Tyr Thr Phe Gly Ser Gly Thr Lys			
	115	120	125	
20	ctg gaa ata aaa cgt acg gtg gct gca cca tct gtc ttc atc ttc ccg			432
	Leu Glu Ile Lys Arg Thr Val Ala Ala Pro Ser Val Phe Ile Phe Pro			
	130	135	140	
25				
	cca tct gat gag cag ttg aaa tct gga act gcc tct gtt gtg tgc ctg			480
30	Pro Ser Asp Glu Gln Leu Lys Ser Gly Thr Ala Ser Val Val Cys Leu			
	145	150	155	160
35	ctg aat aac ttc tat ccc aga gag gcc aaa gta cag tgg aag gtg gat			528
	Leu Asn Asn Phe Tyr Pro Arg Glu Ala Lys Val Gln Trp Lys Val Asp			
	165	170	175	
40				
	aac gcc ctc caa tcg ggt aac tcc cag gag agt gtc aca gag cag gac			576
45	Asn Ala Leu Gln Ser Gly Asn Ser Gln Glu Ser Val Thr Glu Gln Asp			
	180	185	190	
50	agc aag gac agc acc tac agc ctc agc agc acc ctg acg ctg agc aaa			624
	Ser Lys Asp Ser Thr Tyr Ser Leu Ser Ser Thr Leu Thr Leu Ser Lys			
	195	200	205	
55				

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5 gca gac tac gag aaa cac aaa gtc tac gcc tgc gaa gtc acc cat cag 672
Ala Asp Tyr Glu Lys His Lys Val Tyr Ala Cys Glu Val Thr His Gln
210 215 220

10 ggc ctg agc tgc ccc gtc aca aag agc ttc aac agg gga gag tgt tga 720
Gly Leu Ser Ser Pro Val Thr Lys Ser Phe Asn Arg Gly Glu Cys
225 230 235

20 <210> 24

<211> 239

<212> PRT

25 <213> Artificial Sequence

<223> Description of Artificial Sequence: Mouse-human
chimeric antibody (M18D04 L chain)

30 <400> 24

Met Arg Phe Ser Ala Gln Leu Leu Gly Leu Leu Val Leu Trp Ile Pro
35 1 5 10 15

40 Gly Ser Thr Ala Asp Ile Val Met Thr Gln Ala Ala Phe Ser Asn Pro
20 25 30

45 Val Thr Leu Gly Thr Ser Thr Ser Ile Ser Cys Arg Ser Ser Lys Ser
35 40 45

50 Leu Leu His Ser Asn Gly Ile Thr Tyr Leu Tyr Trp Tyr Leu Gln Lys
50 55 60

55

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5	Pro Gly Gln Ser Pro Gln Leu Leu Ile Tyr Gln Met Ser Asn Leu Ala	65	70	75	80
10	Ser Gly Val Pro Asp Arg Phe Ser Ser Ser Gly Ser Gly Thr Asp Phe	85	90	95	
15	Thr Leu Arg Ile Ser Arg Val Glu Ala Glu Asp Val Gly Val Tyr Tyr	100	105	110	
20	Cys Ala Gln Asn Leu Glu Leu Pro Tyr Thr Phe Gly Ser Gly Thr Lys	115	120	125	
25	Leu Glu Ile Lys Arg Thr Val Ala Ala Pro Ser Val Phe Ile Phe Pro	130	135	140	
30	Pro Ser Asp Glu Gln Leu Lys Ser Gly Thr Ala Ser Val Val Cys Leu	145	150	155	160
35	Leu Asn Asn Phe Tyr Pro Arg Glu Ala Lys Val Gln Trp Lys Val Asp	165	170	175	
40	Asn Ala Leu Gln Ser Gly Asn Ser Gln Glu Ser Val Thr Glu Gln Asp	180	185	190	
45	Ser Lys Asp Ser Thr Tyr Ser Leu Ser Ser Thr Leu Thr Leu Ser Lys	195	200	205	
50	Ala Asp Tyr Glu Lys His Lys Val Tyr Ala Cys Glu Val Thr His Gln				
55					

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210

215

220

5

Gly Leu Ser Ser Pro Val Thr Lys Ser Phe Asn Arg Gly Glu Cys

225

230

235

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SEQUENCE LISTING

<110> CHUGAI SEIYAKU KABUSHIKI KAISHA

5 <120> ANTIBODY AGAINST SOLUBLE N-TERMINAL PEPTIDE OR C-TERMINAL PEPTIDE OF
GPC3 PRESENT IN BLOOD

<130> N.94176 GCW

<140> EP 03794236.4

10 <141> 2003-09-04

<150> PCT/JP03/11318

<151> 2003-09-04

15 <150> PCT/JP02/08999

<151> 2002-09-04

<160> 24

<170> PatentIn Ver. 2.1

20 <210> 1

<211> 31

<212> DNA

<213> Artificial Sequence

25 <220>

<223> Description of Artificial Sequence: Synthetic DNA

<400> 1

gatatcatgg ccgggaccgt gcgcaccgcg t 31

30 <210> 2

<211> 31

<212> DNA

<213> Artificial Sequence

35 <220>

<223> Description of Artificial Sequence: Synthetic DNA

<400> 2

gctagctcag tgcaccagga agaagaagca c 31

40 <210> 3

<211> 2300

<212> DNA

<213> Homo sapiens

45 <220>

<221> CDS

<222> (109)..(1851)

<400> 3

cagcacgtct cttgctcctc agggccactg ccaggcttgc cgagtccttg gactgctctc 60

50 gctccggctg ccactctccc gcgctctcct agtcctctgc gaagcagg atg gcc ggg 117

Met Ala Gly

1

acc gtg cgc acc gcg tgc ttg gtg gtg gcg atg ctg ctc agc ttg gac 165

Thr Val Arg Thr Ala Cys Leu Val Val Ala Met Leu Leu Ser Leu Asp

5 10 15

55 ttc ccg gga cag gcg cag ccc ccg ccg ccg ccg gac gcc acc tgt 213

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	Phe	Pro	Gly	Gln	Ala	Gln	Pro	Pro	Pro	Pro	Pro	Pro	Asp	Ala	Thr	Cys	
	20					25				30						35	
	cac	caa	gtc	cgc	tcc	ttc	ttc	cag	aga	ctg	cag	ccc	gga	ctc	aag	tgg	261
5	His	Gln	Val	Arg	Ser	Phe	Phe	Gln	Arg	Leu	Gln	Pro	Gly	Leu	Lys	Trp	
					40					45					50		
	gtg	cca	gaa	act	ccc	gtg	cca	gga	tca	gat	ttg	caa	gta	tgt	ctc	cct	309
	Val	Pro	Glu	Thr	Pro	Val	Pro	Gly	Ser	Asp	Leu	Gln	Val	Cys	Leu	Pro	
				55				60						65			
	aag	ggc	cca	aca	tgc	tgc	tca	aga	aag	atg	gaa	gaa	aaa	tac	caa	cta	357
10	Lys	Gly	Pro	Thr	Cys	Cys	Ser	Arg	Lys	Met	Glu	Glu	Lys	Tyr	Gln	Leu	
			70				75						80				
	aca	gca	cga	ttg	aac	atg	gaa	cag	ctg	ctt	cag	tct	gca	agt	atg	gag	405
	Thr	Ala	Arg	Leu	Asn	Met	Glu	Gln	Leu	Leu	Gln	Ser	Ala	Ser	Met	Glu	
			85				90					95					
	ctc	aag	ttc	tta	att	att	cag	aat	gct	gcg	gtt	ttc	caa	gag	gcc	ttt	453
15	Leu	Lys	Phe	Leu	Ile	Ile	Gln	Asn	Ala	Ala	Val	Phe	Gln	Glu	Ala	Phe	
	100				105					110					115		
	gaa	att	gtt	gtt	cgc	cat	gcc	aag	aac	tac	acc	aat	gcc	atg	ttc	aag	501
	Glu	Ile	Val	Val	Arg	His	Ala	Lys	Asn	Tyr	Thr	Asn	Ala	Met	Phe	Lys	
					120					125				130			
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55	Gly	Arg	Val	Leu	Asp	Lys	Asn	Leu	Asp	Glu	Glu	Gly	Phe	Glu	Ser	Gly
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Asp Cys Gly Asp Asp Glu Asp Glu Cys Ile Ser Gly Asp Gly
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Met Ile Lys Val Lys Asn Gln Leu Arg Phe Leu Ala Glu Leu Ala Tyr
515 520 525
Asp Leu Asp Val Asp Asp Ala Pro Gly Asn Ser Gln Gln Ala Thr Pro
530 535 540
Lys Asp Asn Glu Ile Ser Thr Phe His Asn Leu Gly Asn Val His Ser
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 gtc cag tgt gag gtg caa ctg gtg gag tct ggg gga ggc tta gtg aag 96
 Val Gln Cys Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Lys
 15 20 25 30
 cct gga gga tcc ctg aaa ctc tcc tgt gca gcc tct gga ttc act ttc 144
 Pro Gly Gly Ser Leu Lys Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe
 35 40 45
 agt cgc tat gcc atg tct tgg gtt cgc cag att cca gag aag ata ctg 192
 Ser Arg Tyr Ala Met Ser Trp Val Arg Gln Ile Pro Glu Lys Ile Leu
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 gag tgg gtc gca gcc att gat agt agt ggt ggt gac acc tac tat tta 240
 Glu Trp Val Ala Ala Ile Asp Ser Ser Gly Gly Asp Thr Tyr Tyr Leu
 65 70 75 80
 gac act gtg aag gac cga ttc acc atc tcc aga gac aat gcc aat aat 288
 Asp Thr Val Lys Asp Arg Phe Thr Ile Ser Arg Asp Asn Ala Asn Asn
 25 85 90 95
 acc ctg cac ctg caa atg cgc agt ctg agg tct gag gac aca gcc ttg 336
 Thr Leu His Leu Gln Met Arg Ser Leu Arg Ser Glu Asp Thr Ala Leu
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 tat tac tgt gta aga cag ggg ggg gct tac tgg ggc caa ggg act ctg 384
 Tyr Tyr Cys Val Arg Gln Gly Gly Ala Tyr Trp Gly Gln Gly Thr Leu
 115 120 125
 gtc act gtc tct gca gct agc acc aag ggc cca tcg gtc ttc ccc ctg 432
 Val Thr Val Ser Ala Ala Ser Thr Lys Gly Pro Val Phe Pro Leu
 130 135 140
 gca ccc tcc tcc aag agc acc tct ggg ggc aca gcg gcc ctg ggc tgc 480
 Ala Pro Ser Ser Lys Ser Thr Ser Gly Gly Thr Ala Ala Leu Gly Cys
 145 150 155 160
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 Leu Val Lys Asp Tyr Phe Pro Glu Pro Val Thr Val Ser Trp Asn Ser
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 Ser Gly Leu Tyr Ser Leu Ser Ser Val Val Thr Val Pro Ser Ser Ser
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 Leu Gly Thr Gln Thr Tyr Ile Cys Asn Val Asn His Lys Pro Ser Asn
 210 215 220
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 Thr Lys Val Asp Lys Lys Val Glu Pro Lys Ser Cys Asp Lys Thr His
 225 230 235 240
 50 aca tgc cca ccg tgc cca gca cct gaa ctc ctg ggg gga ccg tca gtc 768
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 245 250 255
 ttc ctc ttc ccc cca aaa ccc aag gac acc ctc atg atc tcc cgg acc 816
 Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met Ile Ser Arg Thr
 55 260 265 270

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	Pro Glu Val Thr Cys Val Val Val Asp Val Ser His Glu Asp Pro Glu	
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	Val Lys Phe Asn Trp Tyr Val Asp Gly Val Glu Val His Asn Ala Lys	
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	aca aag ccg cgg gag gag cag tac aac agc acg tac cgt gtg gtc agc	960
	Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser Thr Tyr Arg Val Val Ser	
	305 310 315 320	
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	325 330 335	
	tgc aag gtc tcc aac aaa gcc ctc cca gcc ccc atc gag aaa acc atc	1056
	Cys Lys Val Ser Asn Lys Ala Leu Pro Ala Pro Ile Glu Lys Thr Ile	
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	cca tcc cgg gat gag ctg acc aag aac cag gtc agc ctg acc tgc ctg	1152
	Pro Ser Arg Asp Glu Leu Thr Lys Asn Gln Val Ser Leu Thr Cys Leu	
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	Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu Ser Asn	
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25	gac ggc tcc ttc ctc ctc tac agc aag ctc acc gtg gac aag agc agg	1296
	Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr Val Asp Lys Ser Arg	
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	Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val Met His Glu Ala Leu	
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50	Glu Trp Val Ala Ala Ile Asp Ser Ser Gly Gly Asp Thr Tyr Tyr Leu	
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55	Thr Leu His Leu Gln Met Arg Ser Leu Arg Ser Glu Asp Thr Ala Leu	
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		Val	His	Ser	Glu	Val	Gln	Leu	Gln	Gln	Ser	Gly	Pro	Glu	Leu	Val	Lys	
5					20					25					30			
		cct	ggg	gct	tca	gtg	aag	ata	tcc	tgc	aag	gct	tct	ggt	tac	tca	ttc	144
		Pro	Gly	Ala	Ser	Val	Lys	Ile	Ser	Cys	Lys	Ala	Ser	Gly	Tyr	Ser	Phe	
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		act	ggc	tac	tac	atg	cac	tgg	gtg	aag	caa	agt	cct	gaa	aag	agc	ctt	192
		Thr	Gly	Tyr	Tyr	Met	His	Trp	Val	Lys	Gln	Ser	Pro	Glu	Lys	Ser	Leu	
10			50					55					60					
		gag	tgg	att	gga	gag	att	aat	cct	agc	act	ggt	ggt	act	acc	tac	aac	240
		Glu	Trp	Ile	Gly	Glu	Ile	Asn	Pro	Ser	Thr	Gly	Gly	Thr	Thr	Tyr	Asn	
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		cag	aag	ttc	aag	gcc	aag	gcc	aca	ttg	act	gta	gac	aaa	tcc	tcc	agc	288
		Gln	Lys	Phe	Lys	Ala	Lys	Ala	Thr	Leu	Thr	Val	Asp	Lys	Ser	Ser	Ser	
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		Thr	Ala	Tyr	Met	Gln	Leu	Lys	Ser	Leu	Thr	Ser	Glu	Asp	Ser	Ala	Val	
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		Tyr	Trp	Gly	Gln	Gly	Thr	Leu	Val	Thr	Val	Ser	Ala	Ala	Ser	Thr	Lys	
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		Gly	Pro	Ser	Val	Phe	Pro	Leu	Ala	Pro	Ser	Ser	Lys	Ser	Thr	Ser	Gly	
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		Gly	Thr	Ala	Ala	Leu	Gly	Cys	Leu	Val	Lys	Asp	Tyr	Phe	Pro	Glu	Pro	
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		Val	Thr	Val	Ser	Trp	Asn	Ser	Gly	Ala	Leu	Thr	Ser	Gly	Val	His	Thr	
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		Val	Thr	Val	Pro	Ser	Ser	Ser	Leu	Gly	Thr	Gln	Thr	Tyr	Ile	Cys	Asn	
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		Lys	Ser	Cys	Asp	Lys	Thr	His	Thr	Cys	Pro	Pro	Cys	Pro	Ala	Pro	Glu	
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		Leu	Leu	Gly	Gly	Pro	Ser	Val	Phe	Leu	Phe	Pro	Pro	Pro	Lys	Lys	Asp	
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45		acc	ctc	atg	atc	tcc	cgg	acc	cct	gag	gtc	aca	tgc	gtg	gtg	gtg	gac	864
		Thr	Leu	Met	Ile	Ser	Arg	Thr	Pro	Glu	Val	Thr	Cys	Val	Val	Val	Asp	
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cca cag gtg tac acc ctg ccc cca tcc cgg gat gag ctg acc aag aac 1152
Pro Gln Val Tyr Thr Leu Pro Pro Ser Arg Asp Glu Leu Thr Lys Asn
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10 cag gtc agc ctg acc tgc ctg gtc aaa ggc ttc tat ccc agc gac atc 1200
Gln Val Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile
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Ala Val Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr
405 410 415
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Thr Pro Pro Val Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys
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Leu Thr Val Asp Lys Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys
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40 Pro Gly Ala Ser Val Lys Ile Ser Cys Lys Ala Ser Gly Tyr Ser Phe
35 40 45
Thr Gly Tyr Tyr Met His Trp Val Lys Gln Ser Pro Glu Lys Ser Leu
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Glu Trp Ile Gly Glu Ile Asn Pro Ser Thr Gly Gly Thr Thr Tyr Asn
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45 Gln Lys Phe Lys Ala Lys Ala Thr Leu Thr Val Asp Lys Ser Ser Ser
85 90 95
Thr Ala Tyr Met Gln Leu Lys Ser Leu Thr Ser Glu Asp Ser Ala Val
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Tyr Tyr Cys Ala Arg Arg Gly Gly Leu Thr Gly Thr Ser Phe Phe Ala
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130 135 140
Gly Pro Ser Val Phe Pro Leu Ala Pro Ser Ser Lys Ser Thr Ser Gly
145 150 155 160
Gly Thr Ala Ala Leu Gly Cys Leu Val Lys Asp Tyr Phe Pro Glu Pro
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5	Val	Thr	Val	Pro	Ser	Ser	Leu
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	Val	Asn	His	Lys	Pro	Ser	Asn
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	Lys	Ser	Cys	Asp	Lys	Thr	His
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10	Leu	Leu	Gly	Gly	Pro	Ser	Val
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	Thr	Leu	Met	Ile	Ser	Arg	Thr
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	Val	Ser	His	Glu	Asp	Pro	Glu
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20	Ala	Pro	Ile	Glu	Lys	Thr	Ile
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	Pro	Gln	Val	Tyr	Thr	Leu	Pro
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	Gln	Val	Ser	Leu	Thr	Cys	Leu
	385						390
25	Ala	Val	Glu	Trp	Glu	Ser	Asn
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	Leu	Thr	Val	Asp	Lys	Ser	Arg
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	Glu Trp Val Ala Ala Ile Asp Ser Asn Gly Gly Thr Thr Tyr Tyr Pro	
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	gac act atg aag gac cga ttc acc att tcc aga gac aat gcc aag aac	288
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35	gaa ctc ctg ggg gga ccg tca gtc ttc ctc ttc ccc cca aaa ccc aag	816
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	Gly Val Glu Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr	
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	aac agc acg tac cgt gtg gtc agc gtc ctc acc gtc ctg cac cag gac	1008
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	Glu	Pro	Gln	Val	Tyr	Thr	Leu	Pro	Pro	Ser	Arg	Asp	Glu	Leu	Thr	Lys	
	370						375					380					
5	aac	cag	gtc	agc	ctg	acc	tgc	ctg	gtc	aaa	ggc	ttc	tat	ccc	agc	gac	1200
	Asn	Gln	Val	Ser	Leu	Thr	Cys	Leu	Val	Lys	Gly	Phe	Tyr	Pro	Ser	Asp	
	385						390				395					400	
	atc	gcc	gtg	gag	tgg	gag	agc	aat	ggg	cag	ccg	gag	aac	aac	tac	aag	1248
	Ile	Ala	Val	Glu	Trp	Glu	Ser	Asn	Gly	Gln	Pro	Glu	Asn	Asn	Tyr	Lys	
					405				410						415		
10	acc	acg	cct	ccc	gtg	ctg	gac	tcc	gac	ggc	tcc	ttc	ttc	ctc	tac	agc	1296
	Thr	Thr	Pro	Pro	Val	Leu	Asp	Ser	Asp	Gly	Ser	Phe	Phe	Leu	Tyr	Ser	
				420					425					430			
	aag	ctc	acc	gtg	gac	aag	agc	agg	tgg	cag	cag	ggg	aac	gtc	ttc	tca	1344
	Lys	Leu	Thr	Val	Asp	Lys	Ser	Arg	Trp	Gln	Gln	Gly	Asn	Val	Phe	Ser	
				435				440					445				
15	tgc	tcc	gtg	atg	cat	gag	gct	ctg	cac	aac	cac	tac	acg	cag	aag	agc	1392
	Cys	Ser	Val	Met	His	Glu	Ala	Leu	His	Asn	His	Tyr	Thr	Gln	Lys	Ser	
				450			455					460					
	ctc	tcc	ctg	tct	ccg	ggt	aaa	tga									1416
	Leu	Ser	Leu	Ser	Pro	Gly	Lys										
	465					470											
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	<211> 471																
	<212> PRT																
	<213> Artificial Sequence																
25	<220>																
	<223> Description of Artificial Sequence: Mouse-human chimeric antibody (M19B11 H chain)																
	<400> 14																
30	Met	Asn	Phe	Gly	Leu	Thr	Leu	Ile	Phe	Leu	Val	Leu	Thr	Leu	Lys	Gly	
	1				5					10					15		
	Val	Gln	Cys	Glu	Val	Gln	Leu	Val	Glu	Ser	Gly	Gly	Asp	Leu	Val	Lys	
				20					25					30			
	Pro	Gly	Gly	Thr	Leu	Lys	Leu	Ser	Cys	Ala	Ala	Ser	Gly	Ser	Thr	Phe	
				35				40					45				
35	Ser	Asn	Tyr	Ala	Met	Ser	Trp	Val	Arg	Gln	Thr	Pro	Glu	Lys	Arg	Leu	
		50				55						60					
	Glu	Trp	Val	Ala	Ala	Ile	Asp	Ser	Asn	Gly	Gly	Thr	Thr	Tyr	Tyr	Pro	
		65				70					75					80	
	Asp	Thr	Met	Lys	Asp	Arg	Phe	Thr	Ile	Ser	Arg	Asp	Asn	Ala	Lys	Asn	
				85					90						95		
40	Thr	Leu	Tyr	Leu	Gln	Met	Asn	Ser	Leu	Arg	Ser	Glu	Asp	Thr	Ala	Phe	
				100					105					110			
	Tyr	His	Cys	Thr	Arg	His	Asn	Gly	Gly	Tyr	Glu	Asn	Tyr	Gly	Trp	Phe	
			115					120					125				
	Ala	Tyr	Trp	Gly	Gln	Gly	Thr	Leu	Val	Thr	Val	Ser	Ala	Ala	Ser	Thr	
				130			135					140					
45	Lys	Gly	Pro	Ser	Val	Phe	Pro	Leu	Ala	Pro	Ser	Ser	Lys	Ser	Thr	Ser	
					150					155						160	
	Gly	Gly	Thr	Ala	Ala	Leu	Gly	Cys	Leu	Val	Lys	Asp	Tyr	Phe	Pro	Glu	
					165					170					175		
	Pro	Val	Thr	Val	Ser	Trp	Asn	Ser	Gly	Ala	Leu	Thr	Ser	Gly	Val	His	
				180					185					190			
50	Thr	Phe	Pro	Ala	Val	Leu	Gln	Ser	Ser	Gly	Leu	Tyr	Ser	Leu	Ser	Ser	
				195				200					205				
	Val	Val	Thr	Val	Pro	Ser	Ser	Ser	Leu	Gly	Thr	Gln	Thr	Tyr	Ile	Cys	
				210			215						220				
55	Asn	Val	Asn	His	Lys	Pro	Ser	Asn	Thr	Lys	Val	Asp	Lys	Lys	Val	Glu	
		225				230					235					240	

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	Pro	Lys	Ser	Cys	Asp	Lys	Thr	His	Thr	Cys	Pro	Pro	Cys	Pro	Ala	Pro	
					245					250					255		
	Glu	Leu	Leu	Gly	Gly	Pro	Ser	Val	Phe	Leu	Phe	Pro	Pro	Lys	Pro	Lys	
5				260					265					270			
	Asp	Thr	Leu	Met	Ile	Ser	Arg	Thr	Pro	Glu	Val	Thr	Cys	Val	Val	Val	
			275					280					285				
	Asp	Val	Ser	His	Glu	Asp	Pro	Glu	Val	Lys	Phe	Asn	Trp	Tyr	Val	Asp	
		290				295						300					
10	Gly	Val	Glu	Val	His	Asn	Ala	Lys	Thr	Lys	Pro	Arg	Glu	Glu	Gln	Tyr	
	305				310						315					320	
	Asn	Ser	Thr	Tyr	Arg	Val	Val	Ser	Val	Leu	Thr	Val	Leu	His	Gln	Asp	
				325						330					335		
	Trp	Leu	Asn	Gly	Lys	Glu	Tyr	Lys	Cys	Lys	Val	Ser	Asn	Lys	Ala	Leu	
			340						345				350				
15	Pro	Ala	Pro	Ile	Glu	Lys	Thr	Ile	Ser	Lys	Ala	Lys	Gly	Gln	Pro	Arg	
		355					360						365				
	Glu	Pro	Gln	Val	Tyr	Thr	Leu	Pro	Pro	Ser	Arg	Asp	Glu	Leu	Thr	Lys	
		370					375					380					
	Asn	Gln	Val	Ser	Leu	Thr	Cys	Leu	Val	Lys	Gly	Phe	Tyr	Pro	Ser	Asp	
	385				390						395					400	
20	Ile	Ala	Val	Glu	Trp	Glu	Ser	Asn	Gly	Gln	Pro	Glu	Asn	Asn	Tyr	Lys	
				405					410						415		
	Thr	Thr	Pro	Pro	Val	Leu	Asp	Ser	Asp	Gly	Ser	Phe	Phe	Leu	Tyr	Ser	
				420					425					430			
	Lys	Leu	Thr	Val	Asp	Lys	Ser	Arg	Trp	Gln	Gln	Gly	Asn	Val	Phe	Ser	
			435				440					445					
25	Cys	Ser	Val	Met	His	Glu	Ala	Leu	His	Asn	His	Tyr	Thr	Gln	Lys	Ser	
		450				455						460					
	Leu	Ser	Leu	Ser	Pro	Gly	Lys										
	465					470											
30	<210> 15																
	<211> 1413																
	<212> DNA																
	<213> Artificial Sequence																
35	<220>																
	<221> CDS																
	<222> (1)..(1410)																
40	<220>																
	<223> Description of Artificial Sequence: Mouse-human chimeric antibody (M18D04 H chain)																
	<400> 15																
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	Met	Glu	Ser	Asn	Trp	Ile	Leu	Pro	Phe	Ile	Leu	Ser	Val	Ala	Ser	Gly	
	1				5				10					15			
45	gtc	tac	tca	gag	gtt	cag	ctc	cag	cag	tct	ggg	act	gtg	ctg	gca	agg	96
	Val	Tyr	Ser	Glu	Val	Gln	Leu	Gln	Ser	Gly	Thr	Val	Leu	Ala	Arg		
				20				25					30				
	cct	ggg	gct	tca	gtg	aag	atg	tcc	tgc	aag	gct	tct	ggc	tac	acc	ttt	144
	Pro	Gly	Ala	Ser	Val	Lys	Met	Ser	Cys	Lys	Ala	Ser	Gly	Tyr	Thr	Phe	
				35			40				45						
50	act	ggc	tac	tgg	atg	cgc	tgg	gta	aaa	cag	agg	cct	gga	cag	ggg	ctg	192
	Thr	Gly	Tyr	Trp	Met	Arg	Trp	Val	Lys	Gln	Arg	Pro	Gly	Gln	Gly	Leu	
		50				55			60								
	gaa	tgg	att	ggc	gct	att	tat	cct	gga	aat	agt	gat	aca	aca	tac	aac	240
	Glu	Trp	Ile	Gly	Ala	Ile	Tyr	Pro	Gly	Asn	Ser	Asp	Thr	Thr	Tyr	Asn	
		65			70				75						80		
55	cag	aag	ttc	aag	ggc	aag	gcc	aaa	ctg	act	gca	gtc	aca	tct	gtc	agc	288

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	Gln	Lys	Phe	Lys	Gly	Lys	Ala	Lys	Leu	Thr	Ala	Val	Thr	Ser	Val	Ser	
					85					90					95		
5	act	gcc	tac	atg	gaa	ctc	agc	agc	ctg	aca	aat	gag	gac	tct	gcg	gtc	336
	Thr	Ala	Tyr	Met	Glu	Leu	Ser	Ser	Leu	Thr	Asn	Glu	Asp	Ser	Ala	Val	
					100				105					110			
	tat	tac	tgt	tca	aga	tcg	ggg	gac	cta	act	ggg	ggg	ttt	gct	tac	tgg	384
	Tyr	Tyr	Cys	Ser	Arg	Ser	Gly	Asp	Leu	Thr	Gly	Gly	Phe	Ala	Tyr	Trp	
					115			120					125				
10	ggc	caa	ggg	act	ctg	gtc	act	gtc	tct	aca	gcc	aaa	gct	agc	acc	aag	432
	Gly	Gln	Gly	Thr	Leu	Val	Thr	Val	Ser	Thr	Ala	Lys	Ala	Ser	Thr	Lys	
		130				135						140					
	ggc	cca	tcg	gtc	ttc	ccc	ctg	gca	ccc	tcc	tcc	aag	agc	acc	tct	ggg	480
	Gly	Pro	Ser	Val	Phe	Pro	Leu	Ala	Pro	Ser	Ser	Lys	Ser	Thr	Ser	Gly	
		145			150						155				160		
15	ggc	aca	gcg	gcc	ctg	ggc	tgc	ctg	gtc	aag	gac	tac	ttc	ccc	gaa	ccg	528
	Gly	Thr	Ala	Ala	Leu	Gly	Cys	Leu	Val	Lys	Asp	Tyr	Phe	Pro	Glu	Pro	
					165				170				175				
	gtg	acg	gtg	tcg	tgg	aac	tca	ggc	gcc	ctg	acc	agc	ggc	gtg	cac	acc	576
	Val	Thr	Val	Ser	Trp	Asn	Ser	Gly	Ala	Leu	Thr	Ser	Gly	Val	His	Thr	
					180			185					190				
20	ttc	ccg	gct	gtc	cta	cag	tcc	tca	gga	ctc	tac	tcc	ctc	agc	agc	gtg	624
	Phe	Pro	Ala	Val	Leu	Gln	Ser	Ser	Gly	Leu	Tyr	Ser	Leu	Ser	Ser	Val	
					195			200					205				
	gtg	acc	gtg	ccc	tcc	agc	agc	ttg	ggc	acc	cag	acc	tac	atc	tgc	aac	672
	Val	Thr	Val	Pro	Ser	Ser	Ser	Leu	Gly	Thr	Gln	Thr	Tyr	Ile	Cys	Asn	
					210		215					220					
25	gtg	aat	cac	aag	ccc	agc	aac	acc	aag	gtg	gac	aag	aaa	gtt	gag	ccc	720
	Val	Asn	His	Lys	Pro	Ser	Asn	Thr	Lys	Val	Asp	Lys	Lys	Val	Glu	Pro	
		225			230					235					240		
	aaa	tct	tgt	gac	aaa	act	cac	aca	tgc	cca	ccg	tgc	cca	gca	cct	gaa	768
	Lys	Ser	Cys	Asp	Lys	Thr	His	Thr	Cys	Pro	Pro	Cys	Pro	Ala	Pro	Glu	
					245				250					255			
30	ctc	ctg	ggg	gga	ccg	tca	gtc	ttc	ctc	ttc	ccc	cca	aaa	ccc	aag	gac	816
	Leu	Leu	Gly	Gly	Pro	Ser	Val	Phe	Leu	Phe	Pro	Pro	Lys	Pro	Lys	Asp	
					260			265					270				
	acc	ctc	atg	atc	tcc	cgg	acc	cct	gag	gtc	aca	tgc	gtg	gtg	gtg	gac	864
	Thr	Leu	Met	Ile	Ser	Arg	Thr	Pro	Glu	Val	Thr	Cys	Val	Val	Val	Asp	
35					275			280					285				
	gtg	agc	cac	gaa	gac	cct	gag	gtc	aag	ttc	aac	tgg	tac	gtg	gac	ggc	912
	Val	Ser	His	Glu	Asp	Pro	Glu	Val	Lys	Phe	Asn	Trp	Tyr	Val	Asp	Gly	
		290				295						300					
	gtg	gag	gtg	cat	aat	gcc	aag	aca	aag	ccg	cgg	gag	gag	cag	tac	aac	960
	Val	Glu	Val	His	Asn	Ala	Lys	Thr	Lys	Pro	Arg	Glu	Glu	Gln	Tyr	Asn	
40		305			310					315					320		
	agc	acg	tac	cgt	gtg	gtc	agc	gtc	ctc	acc	gtc	ctg	cac	cag	gac	tgg	1008
	Ser	Thr	Tyr	Arg	Val	Val	Ser	Val	Leu	Thr	Val	Leu	His	Gln	Asp	Trp	
					325				330						335		
45	ctg	aat	ggc	aag	gag	tac	aag	tgc	aag	gtc	tcc	aac	aaa	gcc	ctc	cca	1056
	Leu	Asn	Gly	Lys	Glu	Tyr	Lys	Cys	Lys	Val	Ser	Asn	Lys	Ala	Leu	Pro	
					340			345					350				
	gcc	ccc	atc	gag	aaa	acc	atc	tcc	aaa	gcc	aaa	ggg	cag	ccc	cga	gaa	1104
	Ala	Pro	Ile	Glu	Lys	Thr	Ile	Ser	Lys	Ala	Lys	Gly	Gln	Pro	Arg	Glu	
					355			360					365				
50	cca	cag	gtg	tac	acc	ctg	ccc	cca	tcc	cgg	gat	gag	ctg	acc	aag	aac	1152
	Pro	Gln	Val	Tyr	Thr	Leu	Pro	Pro	Ser	Arg	Asp	Glu	Leu	Thr	Lys	Asn	
		370				375						380					
	cag	gtc	agc	ctg	acc	tgc	ctg	gtc	aaa	ggc	ttc	tat	ccc	agc	gac	atc	1200
	Gln	Val	Ser	Leu	Thr	Cys	Leu	Val	Lys	Gly	Phe	Tyr	Pro	Ser	Asp	Ile	
					385		390				395				400		
55	gcc	gtg	gag	tgg	gag	agc	aat	ggg	cag	ccg	gag	aac	aac	tac	aag	acc	1248
	Ala	Val	Glu	Trp	Glu	Ser	Asn	Gly	Gln	Pro	Glu	Asn	Asn	Tyr	Lys	Thr	

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                    405                    410                    415
acg cct ccc gtg ctg gac tcc gac ggc tcc ttc ttc ctc tac agc aag 1296
Thr Pro Pro Val Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys
5
                    420                    425                    430
ctc acc gtg gac aag agc agg tgg cag cag ggg aac gtc ttc tca tgc 1344
Leu Thr Val Asp Lys Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys
                    435                    440                    445
tcc gtg atg cat gag gct ctg cac aac cac tac acg cag aag agc ctc 1392
Ser Val Met His Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu
10
                    450                    455                    460
tcc ctg tct ccg ggt aaa tga 1413
Ser Leu Ser Pro Gly Lys
465                    470

<210> 16
<211> 470
<212> PRT
<213> Artificial Sequence

<220>
20 <223> Description of Artificial Sequence: Mouse-human
    chimeric antibody (M18D04 H chain)

<400> 16
Met Glu Ser Asn Trp Ile Leu Pro Phe Ile Leu Ser Val Ala Ser Gly
1 5 10 15
25 Val Tyr Ser Glu Val Gln Leu Gln Gln Ser Gly Thr Val Leu Ala Arg
    20 25 30
Pro Gly Ala Ser Val Lys Met Ser Cys Lys Ala Ser Gly Tyr Thr Phe
    35 40 45
Thr Gly Tyr Trp Met Arg Trp Val Lys Gln Arg Pro Gly Gln Gly Leu
    50 55 60
30 Glu Trp Ile Gly Ala Ile Tyr Pro Gly Asn Ser Asp Thr Thr Tyr Asn
    65 70 75 80
Gln Lys Phe Lys Gly Lys Ala Lys Leu Thr Ala Val Thr Ser Val Ser
    85 90 95
Thr Ala Tyr Met Glu Leu Ser Ser Leu Thr Asn Glu Asp Ser Ala Val
35 Tyr Tyr Cys Ser Arg Ser Gly Asp Leu Thr Gly Gly Phe Ala Tyr Trp
    100 105 110
    115 120 125
Gly Gln Gly Thr Leu Val Thr Val Ser Thr Ala Lys Ala Ser Thr Lys
    130 135 140
40 Gly Pro Ser Val Phe Pro Leu Ala Pro Ser Ser Lys Ser Thr Ser Gly
    145 150 155 160
Gly Thr Ala Ala Leu Gly Cys Leu Val Lys Asp Tyr Phe Pro Glu Pro
    165 170 175
Val Thr Val Ser Trp Asn Ser Gly Ala Leu Thr Ser Gly Val His Thr
    180 185 190
45 Phe Pro Ala Val Leu Gln Ser Ser Gly Leu Tyr Ser Leu Ser Ser Val
    195 200 205
Val Thr Val Pro Ser Ser Ser Leu Gly Thr Gln Thr Tyr Ile Cys Asn
    210 215 220
Val Asn His Lys Pro Ser Asn Thr Lys Val Asp Lys Lys Val Glu Pro
    225 230 235 240
50 Lys Ser Cys Asp Lys Thr His Thr Cys Pro Pro Cys Pro Ala Pro Glu
    245 250 255
Leu Leu Gly Gly Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp
    260 265 270
Thr Leu Met Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp
    275 280 285
55 Val Ser His Glu Asp Pro Glu Val Lys Phe Asn Trp Tyr Val Asp Gly

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290          295          300
Val Glu Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr Asn
305          310          315          320
5  Ser Thr Tyr Arg Val Val Ser Val Leu Thr Val Leu His Gln Asp Trp
          325          330          335
Leu Asn Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Ala Leu Pro
          340          345          350
Ala Pro Ile Glu Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu
          355          360          365
10 Pro Gln Val Tyr Thr Leu Pro Pro Ser Arg Asp Glu Leu Thr Lys Asn
          370          375          380
Gln Val Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile
385          390          395          400
Ala Val Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr
          405          410          415
15 Thr Pro Pro Val Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys
          420          425          430
Leu Thr Val Asp Lys Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys
          435          440          445
Ser Val Met His Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu
          450          455          460
20 Ser Leu Ser Pro Gly Lys
          465          470

<210> 17
<211> 717
25 <212> DNA
    <213> Artificial Sequence

<220>
<221> CDS
30 <222> (1)..(714)

<220>
<223> Description of Artificial Sequence: Mouse-human
      chimeric antibody (M3C11 L chain)

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    Met Ser Pro Ala Gln Phe Leu Phe Leu Val Leu Trp Ile Arg Glu
      1          5          10          15
    acc aac ggt gat gtt gtg atg acc cag act cca ctc act ttg tcg gtt 96
    Thr Asn Gly Asp Val Val Met Thr Gln Thr Pro Leu Thr Leu Ser Val
      20          25          30
    acc att gga caa cca gcc tcc atc tct tgc aag tca agt cag agc ctc 144
    Thr Ile Gly Gln Pro Ala Ser Ile Ser Cys Lys Ser Ser Gln Ser Leu
      35          40          45
    tta gat agt gat gga aag aca tat ttg aat tgg ttg tta cag agg cca 192
    Leu Asp Ser Asp Gly Lys Thr Tyr Leu Asn Trp Leu Leu Gln Arg Pro
      50          55          60
    ggc cag tct cca aag cgc cta atc tat ctg gtg tct aaa ttg gac tct 240
    Gly Gln Ser Pro Lys Arg Leu Ile Tyr Leu Val Ser Lys Leu Asp Ser
      65          70          75          80
    gga gcc cct gac agg ttc act ggc agt gga tca ggg aca gat ttc aca 288
    Gly Ala Pro Asp Arg Phe Thr Gly Ser Gly Ser Gly Thr Asp Phe Thr
      85          90          95
    ctg aaa atc agt aga gtg gag gct gag gat ttg gga att tat tat tgc 336
    Leu Lys Ile Ser Arg Val Glu Ala Glu Asp Leu Gly Ile Tyr Tyr Cys
      100          105          110
    tgg caa ggt aca cat ttt ccg ctc acg ttc ggt gct ggg acc aag ctg 384
    Trp Gln Gly Thr His Phe Pro Leu Thr Phe Gly Ala Gly Thr Lys Leu
      115          120          125          130

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      115      120      125
gag ctg aaa cgt acg gtg gct gca cca tct gtc ttc atc ttc ccg cca 432
Glu Leu Lys Arg Thr Val Ala Ala Pro Ser Val Phe Ile Phe Pro Pro
      130      135      140
5  tct gat gag cag ttg aaa tct gga act gcc tct gtt gtg tgc ctg ctg 480
   Ser Asp Glu Gln Leu Lys Ser Gly Thr Ala Ser Val Val Cys Leu Leu
      145      150      155      160
   aat aac ttc tat ccc aga gag gcc aaa gta cag tgg aag gtg gat aac 528
   Asn Asn Phe Tyr Pro Arg Glu Ala Lys Val Gln Trp Lys Val Asp Asn
      165      170      175
10  gcc ctg caa tcg ggt aac tcc cag gag agt gtc aca gag cag gac agc 576
   Ala Leu Gln Ser Gly Asn Ser Gln Glu Ser Val Thr Glu Gln Asp Ser
      180      185      190
   aag gac agc acc tac agc ctc agc acc ctg acg ctg agc aaa gca 624
   Lys Asp Ser Thr Tyr Ser Leu Ser Ser Thr Leu Thr Leu Ser Lys Ala
      195      200      205
   gac tac gag aaa cac aaa gtc tac gcc tgc gaa gtc acc cat cag ggc 672
   Asp Tyr Glu Lys His Lys Val Tyr Ala Cys Glu Val Thr His Gln Gly
      210      215      220
   ctg agc tcg ccc gtc aca aag agc ttc aac agg gga gag tgt tga 717
20  Leu Ser Ser Pro Val Thr Lys Ser Phe Asn Arg Gly Glu Cys
      225      230      235

<210> 18
<211> 238
<212> PRT
25 <213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Mouse-human
      chimeric antibody (M3C11 L chain)

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   Thr Asn Gly Asp Val Val Met Thr Gln Thr Pro Leu Thr Leu Ser Val
      20      25      30
35  Thr Ile Gly Gln Pro Ala Ser Ile Ser Cys Lys Ser Ser Gln Ser Leu
      35      40      45
   Leu Asp Ser Asp Gly Lys Thr Tyr Leu Asn Trp Leu Leu Gln Arg Pro
      50      55      60
   Gly Gln Ser Pro Lys Arg Leu Ile Tyr Leu Val Ser Lys Leu Asp Ser
      65      70      75      80
40  Gly Ala Pro Asp Arg Phe Thr Gly Ser Gly Ser Gly Thr Asp Phe Thr
      85      90      95
   Leu Lys Ile Ser Arg Val Glu Ala Glu Asp Leu Gly Ile Tyr Tyr Cys
      100      105      110
   Trp Gln Gly Thr His Phe Pro Leu Thr Phe Gly Ala Gly Thr Lys Leu
      115      120      125
45  Glu Leu Lys Arg Thr Val Ala Ala Pro Ser Val Phe Ile Phe Pro Pro
      130      135      140
   Ser Asp Glu Gln Leu Lys Ser Gly Thr Ala Ser Val Val Cys Leu Leu
      145      150      155      160
   Asn Asn Phe Tyr Pro Arg Glu Ala Lys Val Gln Trp Lys Val Asp Asn
      165      170      175
50  Ala Leu Gln Ser Gly Asn Ser Gln Glu Ser Val Thr Glu Gln Asp Ser
      180      185      190
   Lys Asp Ser Thr Tyr Ser Leu Ser Ser Thr Leu Thr Leu Ser Lys Ala
      195      200      205
   Asp Tyr Glu Lys His Lys Val Tyr Ala Cys Glu Val Thr His Gln Gly
55  210      215      220

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Leu Ser Ser Pro Val Thr Lys Ser Phe Asn Arg Gly Glu Cys
 225 230 235

5 <210> 19
 <211> 717
 <212> DNA
 <213> Artificial Sequence

10 <220>
 <221> CDS
 <222> (1)..(714)

15 <220>
 <223> Description of Artificial Sequence: Mouse-human
 chimeric antibody (M1E07 L chain)

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      165              170              175
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Claims

1. An antibody against an N-terminal peptide of GPC 3.
- 5 2. The antibody claimed in Claim 1 wherein the N-terminal peptide of GPC 3 is a secreted form of a peptide found in blood.
3. The antibody claimed in Claim 2 wherein the N-terminal peptide of GPC 3 is a peptide comprising amino acid residues 1-374 of GPC 3 or a peptide comprising amino acid residues 1-358 of GPC 3.
- 10 4. The antibody claimed in Claim 3 wherein the N-terminal peptide of GPC 3 is a peptide comprising amino acid residues 1-358 of GPC 3.
5. The antibody claimed in any one of Claims 1-4 which is a monoclonal antibody.
- 15 6. The antibody claimed in Claim 1 which is immobilized to an insoluble support.
7. The antibody claimed in Claim 1 which is labeled with a labeling material.
- 20 8. An antibody against a C-terminal peptide of GPC 3.
9. The antibody claimed in Claim 8 wherein the C-terminal peptide of GPC 3 is a peptide comprising amino acid residues 359-580 of GPC 3 or a peptide comprising amino acid residues 375-580 of GPC 3.
- 25 10. The antibody claimed in Claim 8 wherein the C-terminal peptide of GPC 3 is a peptide comprising amino acid residues 359-580 of GPC 3.
11. The antibody claimed in any one of Claims 8-10 which is a monoclonal antibody.
- 30 12. The antibody claimed in any one of Claims 8-10 which is a chimera antibody.
13. The antibody claimed in any one of Claims 8-10 which is a cytotoxic antibody.
14. A cell disrupting agent comprising the antibody claimed in any one of Claims 7-13.
- 35 15. The cell disrupting agent claimed in Claim 14 wherein the cell is a cancer cell.
16. An anti-cancer agent comprising the antibody claimed in any one of Claims 8-13.
- 40 17. A method for inducing cytotoxicity comprising contacting a cell with the antibody claimed in any one of Claims 8-13.
18. The method claimed in Claim 17 wherein the cell is a cancer cell.

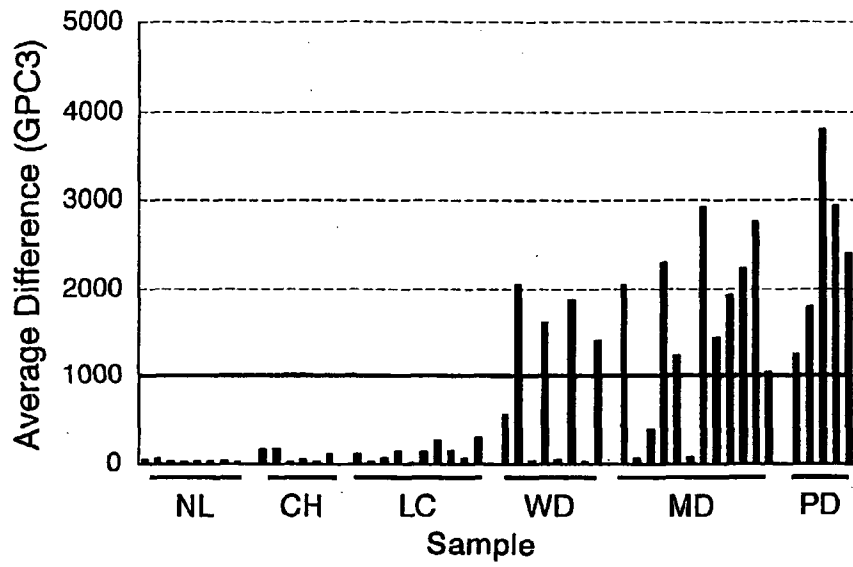
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Fig. 1

A.



B.

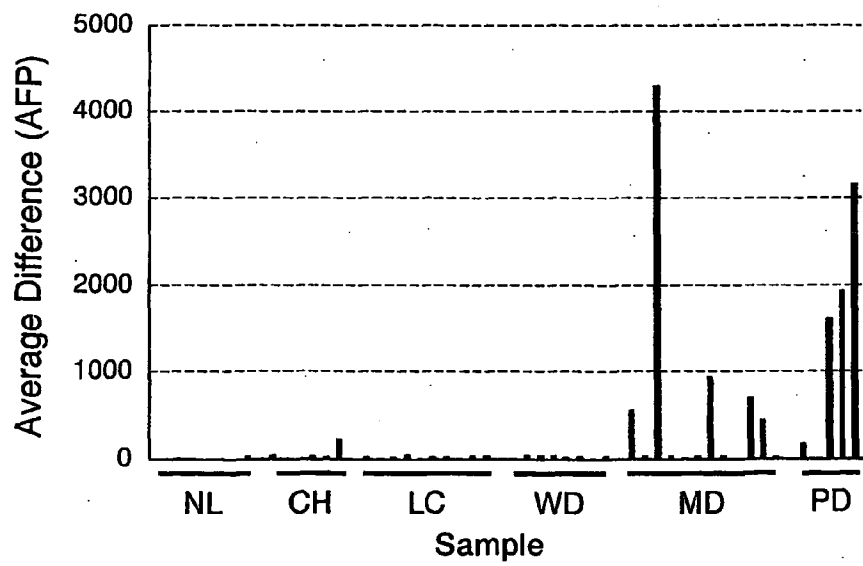


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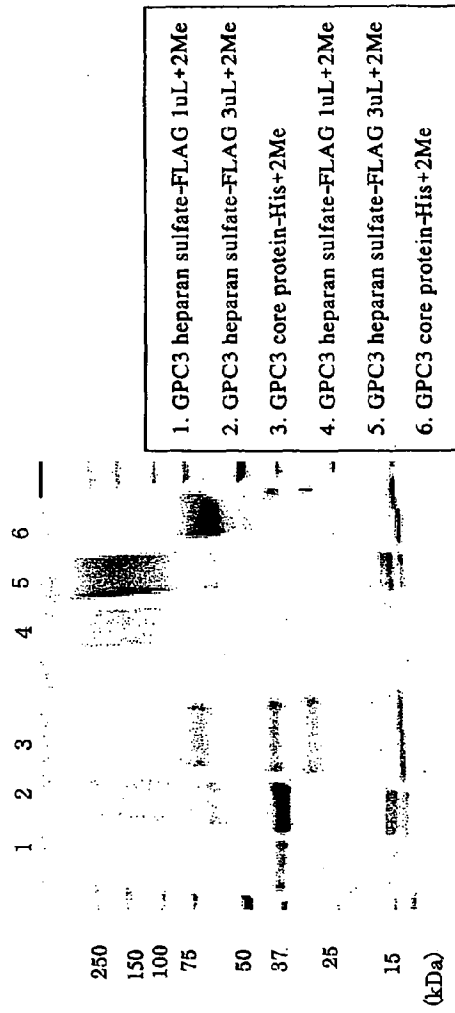


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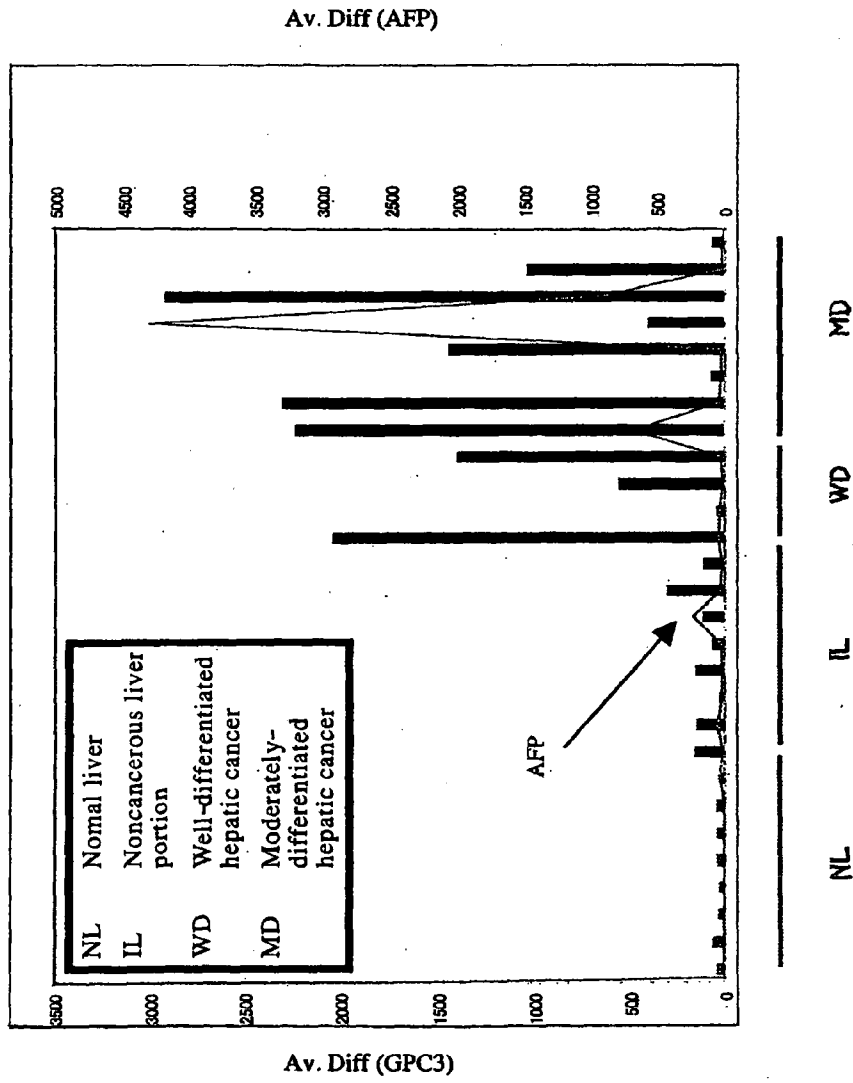


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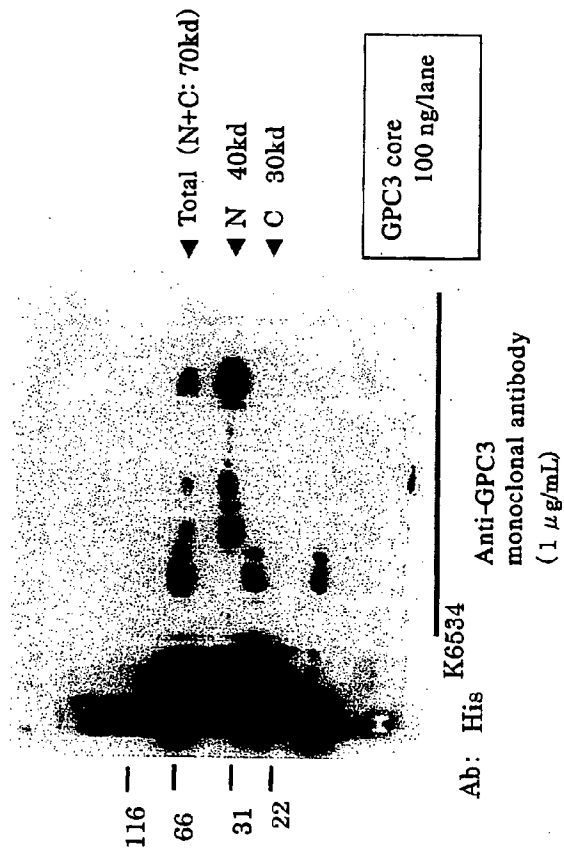


Fig. 5

OD measurement

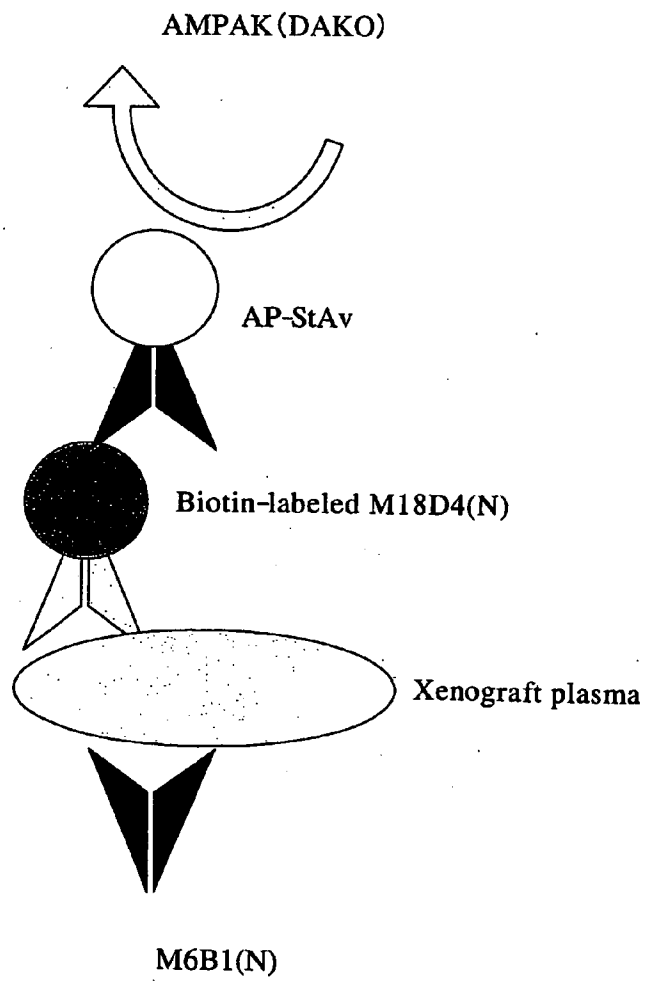


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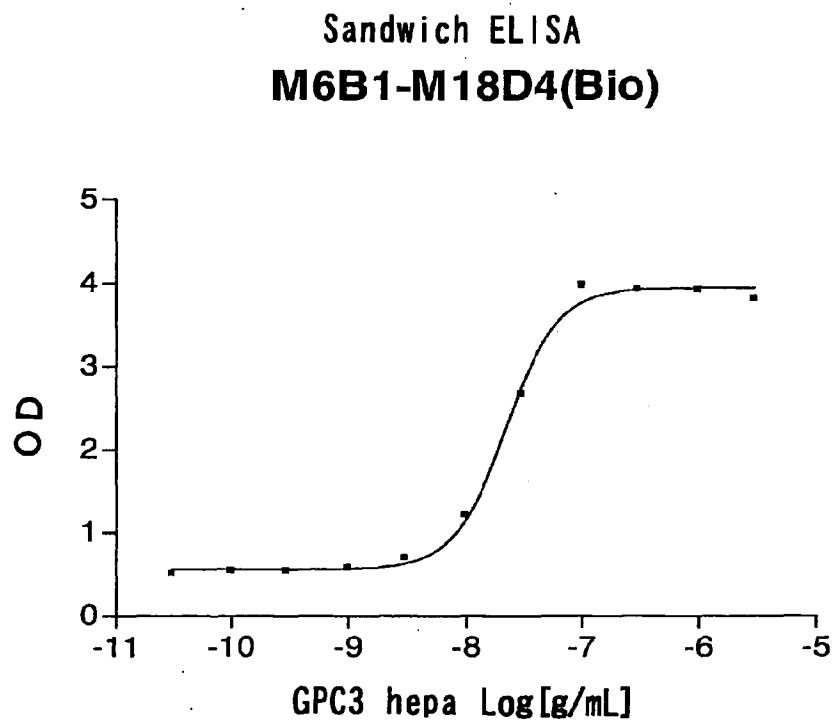


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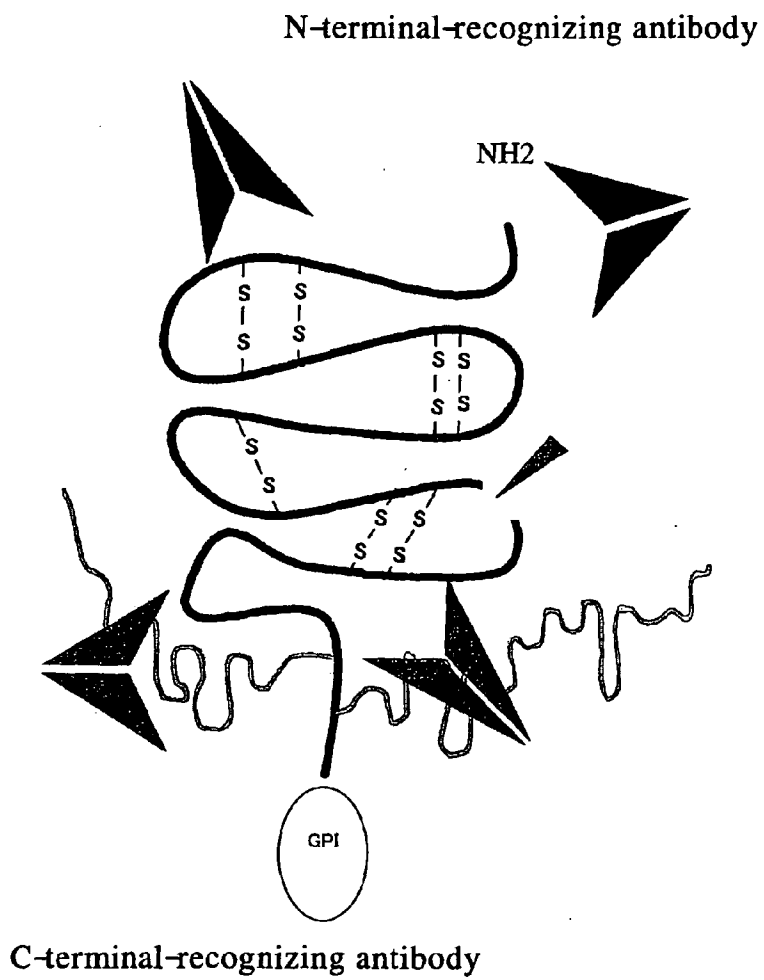


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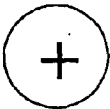
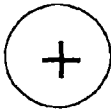
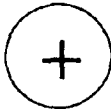
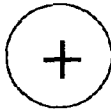
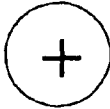
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Fig. 9

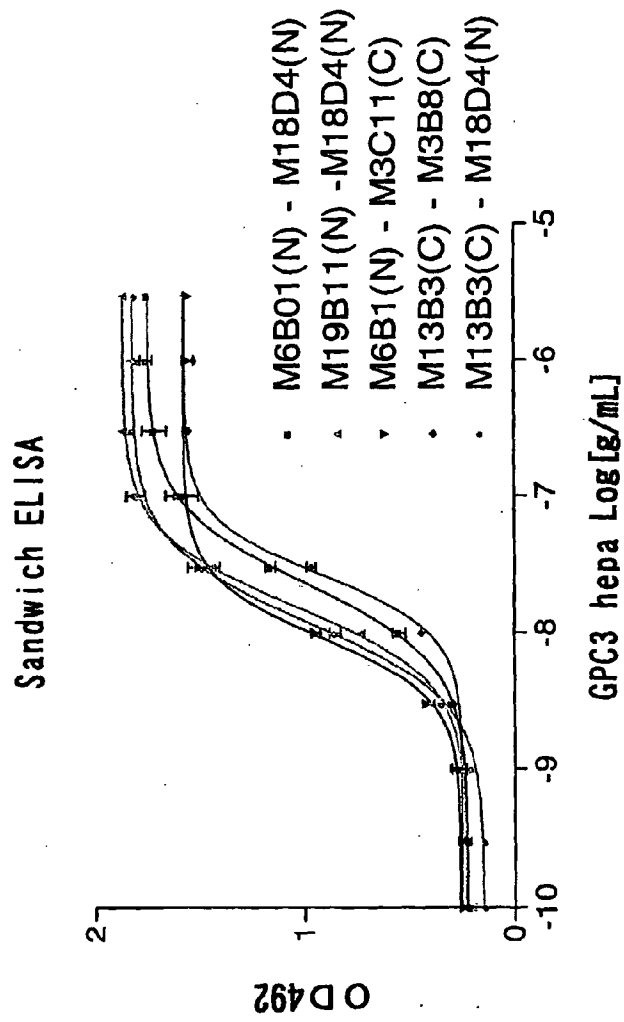


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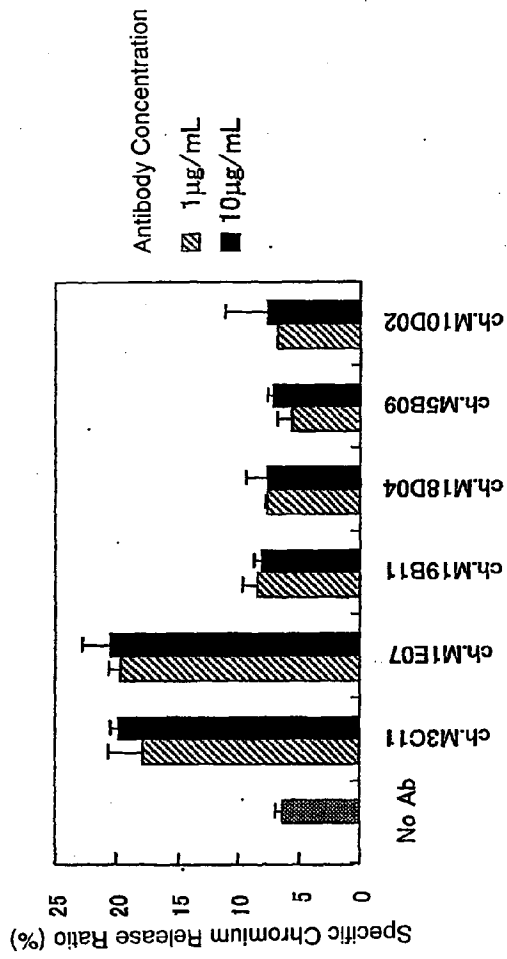
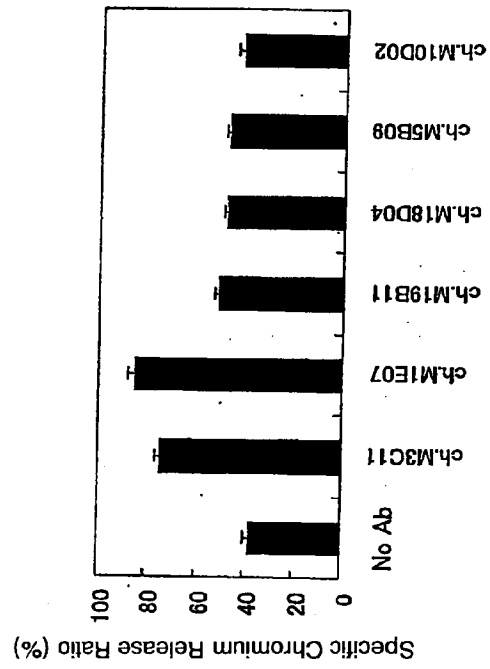


Fig. 11



INTERNATIONAL SEARCH REPORT

International application No.

PCT/JP03/11318

A. CLASSIFICATION OF SUBJECT MATTER Int.Cl ⁷ C12N15/06, C07K16/18 According to International Patent Classification (IPC) or to both national classification and IPC		
B. FIELDS SEARCHED Minimum documentation searched (classification system followed by classification symbols) Int.Cl ⁷ C12N15/00-15/90, C07K16/00-16/46 Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched Electronic data base consulted during the international search (name of data base and, where practicable, search terms used) BIOSIS/MEDLINE/WPIDS (STN), JSTPlus (JOIS)		
C. DOCUMENTS CONSIDERED TO BE RELEVANT		
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	CAPPURO M.I. et al., 'Overexpression of glypican-3 in human hepatocellular carcinomas determined by immunohistochemistry using a monoclonal antibody', Proceeding of the American Association for Cancer Research Annual Meeting, March 2002, Vol.43, page 219	1-13
P,X	WO 03/000883 A1 (Chugai Pharmaceutical Co., Ltd., Hiroyuki ABURAYA), 03 January, 2003 (03.01.03), (Family: none)	1-16
P,X	WO 03/010336 A2 (DEBUSCHWITZ S., JOBST J., KAISER S.), 06 February, 2003 (06.02.03), Page 21, Accession Nr.L47, 125.1 (Family: none)	1-13
<input checked="" type="checkbox"/> Further documents are listed in the continuation of Box C. <input type="checkbox"/> See patent family annex.		
* Special categories of cited documents: "A" document defining the general state of the art which is not considered to be of particular relevance "E" earlier document but published on or after the international filing date "I" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified) "O" document referring to an oral disclosure, use, exhibition or other means "P" document published prior to the international filing date but later than the priority date claimed		"I" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention "X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone "Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art "&" document member of the same patent family
Date of the actual completion of the international search 23 October, 2003 (23.10.03)		Date of mailing of the international search report 04 November, 2003 (04.11.03)
Name and mailing address of the ISA/ Japanese Patent Office		Authorized officer
Facsimile No.		Telephone No.

Form PCT/ISA/210 (second sheet) (July 1998)

INTERNATIONAL SEARCH REPORT

International application No.

PCT/JP03/11318

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
P,X	MIDORIKAWA, Y. et al., 'Glypican-3, overexpressed in hepato-cellular carcinoma, modulates FGF2 and BMP-7 signaling', International Journal of Cancer, 10 February, 2003 (10.02.03), Vol.103, No.4, pages 455 to 465	1-13
P,X	SUNG Y.K. et al., 'Glypican-3 is overexpressed in human hepatocellular carcinoma', Cancer Science, March 2003, Vol.94, No.3, pages 259 to 262	1-13
P,X	CAPURRO M. et al, 'Glypican-3: a novel serum and histochemical marker for hepatocellular carcinoma', GASTROENTEROLOGY, July 2003, 125(1), 89-97	1-13
A	LAGE H. et al., 'Cloning and characterization of human cDNAs encoding a protein with high homology to rat intestinal development protein OCI-5', Gene, 188(1997), 151-156	1-16

Form PCT/ISA/210 (continuation of second sheet) (July 1998)

INTERNATIONAL SEARCH REPORT

International application No.

PCT/JP03/11318

Box I Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

- 1.
- ☒
- Claims Nos.: 17, 18

because they relate to subject matter not required to be searched by this Authority, namely:

Claims 17 and 18 involve methods for treatment of the human body by therapy and diagnostic methods and thus relate to a subject matter which this International Searching Authority is not required, under the provisions of Article 17(2)(a)(i) of the PCT and Rule 39.1(iv) of the Regulations under the PCT, to search.

- 2.
- ☐
- Claims Nos.:

because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

- 3.
- ☐
- Claims Nos.:

because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:
4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest ☐ The additional search fees were accompanied by the applicant's protest.
☐ No protest accompanied the payment of additional search fees.